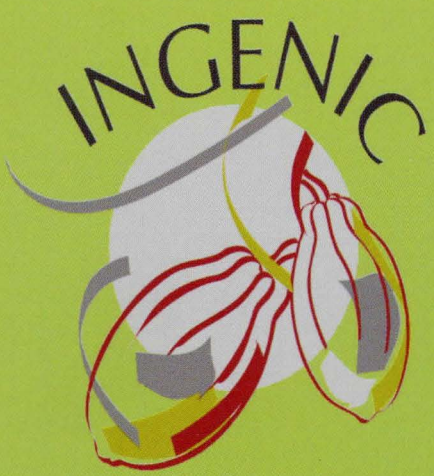
Proceedings of the

International Workshop on Cocoa Breeding for Improved Production Systems



19th-21st October 2003, Accra, Ghana

Editors: F. Bekele, M.J. End and A.B. Eskes

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International Workshop

on

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INGENIC is the International Group for Genetic Improvement of Cocoa. The Group was created in 1994 and operates as an independent group sponsored by different institutions. It promotes the exchange of information and international collaboration on cocoa genetics and improvement of cocoa planting materials. At the occasion of the Accra Workshop, in October 2003, the "Study Group in the Molecular Biology of Cocoa" (INGENIC Mol-Biol) was formed, which will function as a special sub-group of INGENIC. To join this group you can send a blanc e-mail to join-ingenic-mol-biol@lists.cas.psu.edu.

INGENIC has set up a website (http:guiltinanlab.cas.psu.edu/ingenic.htm) and an e-mail list serves (ingenic-email@lists.cas.psu.edu), to which you can send messages for distribution to all e-mail correspondents of INGENIC. Instructions are available at the website. Further Information on INGENIC and copies of INGENIC publications can be obtained from the INGENIC Secretariat (Dr. Michelle J. End, c/o BCCCA, 6 Catherine Street, London, WC2B 5JJ, U.K., e-mail: michelle.end@bccca.org.uk) or accessed at the INGENIC website.

The Ghana Cocoa Board (COCOBOD) is a statutory public body. It is the Central Administrative body of the Institutions and Organisations concerned with various operating and servicing aspects of the Ghana Cocoa Industry. The Cocoa Research Institute of Ghana is a major institution under the COCOBOD.

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for financial support for the Workshop.

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for sponsoring participation of several researchers and invited speakers.

Cocoa Research Institute of Ghana (CRIG), Ghana,

for general support and for organisation of the Workshop.

Ghana Cocoa Board (Cocobod), Ghana,

for general support and sponsoring the joint cocktail and dinner.

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for support to the organisation of this Workshop in conjunction with the 14th International Cocoa Research Conference.

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for sponsoring the participation of an invited speaker and co-sponsoring the Workshop dinner.

INGENIC wishes to thank furthermore the following organisations for their more general support to INGENIC activities and to the INGENIC Committee members: WCF (World Cocoa Foundation), BCCCA, CIRAD (Centre de Coopération Internationale en Recherches Agronomiques pour le Développement, France), CPA, CRU (Cocoa Research Unit, Trinidad and Tobago), CRIG, MCB (Malaysian Cocoa Board, Malaysia), Pennsylvania State University (USA), and UESC (Universidade Estadual de Santa Cruz, Ilheus, Bahia, Brazil).

Workshop Organising Committees

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International Committee (INGENIC)

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PREFACE

This is the fourth International Workshop to be organised by INGENIC, the International Group for the Genetic Improvement of Cocoa. The INGENIC Workshops provide an important opportunity for cocoa breeders to exchange views amongst themselves and with experts from related fields. INGENIC decided to organise its fourth workshop on 'Cocoa Breeding for Improved Production Systems' following a recommendation of the INGENIC General Assembly (Malaysia, October 2000). INGENIC is most grateful to the Cocoa Producers' Alliance and to the Ghana Cocoa Board for allowing this workshop to be held on the occasion of the 14th International Cocoa Research Conference, October 2003, Accra, Ghana.

The theme of the current workshop is of great importance, because average productivity of cocoa is still very low and progress in improving efficiency of production is more limited than in other tree crops, such as apples and pears. Low yielding varieties may be so because of low yield efficiency or because of poor adaptation to the environment. Low yields are sometimes obtained even under highly favourable growing conditions, which is an apparent paradox. Cocoa breeding to date might have overemphasised vegetative growth, which is important for rapid establishment of young trees, but which may be a disadvantage for adult plantations due to strong interplant competition and difficult management of the crop. Yield decline with age of the plantation is a common phenomenon. The continuous spread of devastating pathogens and pests also implies the need for lower cocoa canopies, which can be more easily managed. These important challenges need to be faced by breeders and agronomists in order to assist cocoa farmers.

These issues were translated into the following questions that were dealt with in the workshop sessions:

- How to select superior mother trees in heterogeneous progenies for more productive clone or hybrid varieties?
- What are the factors affecting yield x vigour relationships in cocoa?
- What are the needs and possibilities of developing more compact cocoa varieties?
- What do we know about genotype by environment interactions in cocoa, including the effect of high density planting on long-term cocoa yield?

The participation of Dr. Chris Atkinson, of the Horticultural Research Institute at East Malling in the UK, was very useful to inform about development of dwarfing rootstocks and other growth control measures in temperate fruit tree crops. The objective of this work was to "obtain smaller and more easily manageable trees" which is also an important aspect of our discussions on cocoa.

We were glad for the active participation of cocoa physiologists and agronomists together with cocoa breeders and geneticists to discuss the traits that cocoa varieties must possess to yield more efficiently and to be adapted to different methods of cocoa cultivation, with respect to the environment and to the needs of cocoa farmers.

The editors were pleased to receive full papers of good quality relatively soon after the Workshop. The editing of these papers, including the feedback between the editors and the authors, was necessarily more time-consuming.

INGENIC is engaged in organising its fifth workshop, which will most likely coincide with the 15th International Cocoa Research Conference, to be held in Costa Rica in 2006. The proposed theme of the workshop is "Cocoa Breeding to Meet Farmers' Needs". It will probably embrace an assessment of the impact of breeding programmes, participatory breeding efforts and methods used in practical breeding programmes.

The editors of the Workshop Proceedings January 2005

Frances Bekele
Michelle End
Albertus Eskes

WELCOMING ADDRESSES

Presented at the Joint-Opening of the 4th INGENIC Workshop and the INCOPED 4th International Seminar

Mr. Kwame Sarpong
Chief Executive, Ghana Cocoa Board

The Secretary General of the Cocoa Producers' Alliance (COPAL),
The Chairman of the Scientific Committee of COPAL and the International Permanent Working Group for Cocoa Pests and Diseases (INCOPED),
The Chairman for the International Group for Genetic Improvement of Cocoa (INGENIC),
Deputy Chief Executives of Ghana Cocoa Board,
Executive Director of the Cocoa Research Institute of Ghana,
Distinguished Scientists,
Ladies and Gentlemen.

It is my greatest pleasure and honour to welcome, on behalf of the Government of Ghana, the Local Organising Committees of the International Permanent Working Group for Cocoa Pests and Diseases (INCOPED) 4th Seminar and the International Group for Genetic Improvement of Cocoa (INGENIC) 4th Workshop from various parts of the world to Ghana. It is worth noting that INCOPED was conceived and born in Ghana at the initiative of the Cocoa Research Institute of Ghana and that the 1st Seminar was held in Accra in November, 1995 under the auspices of the Ghana Cocoa Board (COCOBOD).

Incidentally, INGENIC was born in Yamoussoukro, in neighbouring Côte d'Ivoire in 1993. Since then, the two groups have met to discuss different themes in different countries and continents, which have impacted, positively on cocoa production worldwide. Thus hosting these two events here in Ghana today after the just ended 14th International Cocoa Research Conference (ICRC) is significant.

The primary aim of the two groups is to exchange information, through newsletters, seminars/workshops, and also to promote collaboration in the fields of cocoa crop protection and genetics. Dear delegates, you will all agree with me that of all the factors detrimental to cocoa production, the highest losses worldwide are attributed to pests and diseases. This phenomenon is further aggravated by the unstable world cocoa prices. The search for more economic and environmentally friendly forms of pest and disease management practices has thus become imperative. High yielding cocoa trees that are well adapted to their environments can moderate these management methods.

Thus, there could not have been better themes than those chosen for the INCOPED 4th International Seminar and the 4th INGENIC Workshop *i.e.* "Dealing with Pressing Crop Protection Problems" and "Cocoa Breeding for Improved Production Systems" respectively. The two themes complement each other and are very appropriate, particularly in this era when our governments are committed to taking positive steps in cocoa production to alleviate poverty associated with farmers.

The topics selected for the Seminar and Workshop, I believe, will examine recent developments in cocoa production worldwide and allow for an exchange of information for the benefit of producers and manufacturers, as well as consumers. It is my expectation that the ultimate beneficiary from your discussions will be the farmer who will be encouraged to sustain the production of cocoa - "the food of the gods".

On behalf of the Government of Ghana and the Ghana Cocoa Board, I once again welcome you all to the Seminar and Workshop and hope that you will have successful deliberations and enjoy your stay in Ghana.

Thank you.

Hope Sona Ebai
Secretary General of Cocoa Producers' Alliance

Mr. Chairman, Sir,
The Chief Executive, Ghana Cocoa Board
The Chairmen of INCOPED and INGENIC
The Deputy Chief Executive, Ghana Cocoa Board
Eminent Scientists and Participants
Ladies and Gentlemen,

I am indeed very happy to be here with you all today as you begin your deliberations in your respective groups of INGENIC and INCOPED. The fact that you are meeting alongside the meeting of the 14th International Cocoa Research Conference of the Alliance is no coincidence. INCOPED and INGENIC are progenies of the Scientific Research Committee of the Alliance. About eight (8) years later of course you have become groups that are independent and of great renown. I am very excited about the work that's going on; Breeders are getting more and more comfortable with the classification of the materials they use; thanks to new biotechnological applications in the area of Genetics. Time lags for producing improved planting materials have been reduced. This is exciting.

As end consumers become more and more sophisticated and we develop the quantitative techniques to measure infinitesimal levels of undesirables, industry and indeed the whole cocoa economy will continue to be under enormous pressure to deliver products that are not only delicious but also safe, nutritious and produced responsibly. This is already translating into the need for less and less chemical control agents for pests and diseases and more use of biological and natural agents. We are happy to note great strides and new horizons opening in this field.

Some of you in pest and disease control have attempted to classify these in terms of their global impact, usually translated into percentages of crop loss. Even though this is in line with the new thinking of a global approach to problem solving, we hope this does not translate directly into priorities attributed to these problems. Let me elucidate. *Phytophthora* is pan-tropical, but it only accounts for 37% global crop loss because it happens to be prevalent in a zone that accounts for over 65% of world production. *Crinipellis* may, on the other hand, account for 18% global crop loss but this for Brazil represents 75% crop loss. There is therefore a need to rationalise national, regional and global priorities.

You may be aware of the global co-ordination initiative towards a sustainable cocoa economy. The networking you have developed in the two groups should serve to rapidly collect and disseminate technical and scientific information permitting us to create synergies and quickly identify gaps in on-going research endeavours.

The themes and papers to be discussed during the next two days fall perfectly in line with the search for solutions to problems facing cocoa thus enabling us to achieve a sustainable world cocoa economy. We can only but encourage you to continue in your endeavours. We hope that your deliberations lead to results that can be exploited by farmers in their efforts to protect their cocoa biomass, - this "food of the gods" with so many enemies, through the use of better performing vegetative material.

Finally we hope that through you, your colleagues in Agronomy and Chemistry and Technology would be encouraged to formalise their own working groups so that "Long live international scientific co-operation".

I thank you very much indeed for your kind attention.

Joao Louis Pereira
Chairman of the International Permanent Working Group for Cocoa Pests and Diseases (INCOPED)

Mr. Chairman
The Chief Executive, Ghana Cocoa Board
Deputy Chief Executive, Ghana Cocoa Board (A & QC)
The General Secretary, Cocoa Producers' Alliance
The Chairmen of the National Organising Committees for the INCOPED 4th International Seminar & 4th INGENIC Workshop
Fellow Scientists, Distinguished Guests, Ladies and Gentlemen

Having this 4th International Permanent Working Group for Cocoa Pests and Diseases Seminar, here in Accra, Ghana is of very special significance to all of us present.

In November 1995, in this city, a Pests and Diseases Meeting was organised by scientists of the Cocoa Research Institute of Ghana. Besides the success of that meeting, they also had the commendable vision of proposing the formation of an international cocoa pests and diseases group, aimed at meeting the demanding needs in international cooperation, in this specialised area. Therefore, I am sure all of you would appreciate the pleasure it gives me to return to Accra, a venue of historic importance to INCOPED.

In the following year, I followed this up by scheduling a meeting when organising the 12th ICRC in Brazil, taking advantage of the presence of thirty-three cocoa specialists in crop protection from fourteen countries. The Terms of Reference of the Working Group were presented for analysis with a request for acceptance. This formalised the operation of INCOPED.

It is through our previous Seminars and publication of the proceedings, I believe, we have, and continue to make, our contributions to a singular most important area of study that relates to the economic sustainability of cocoa cultivation. In fact, I do not feel the need for modesty, in saying that when the Global Coordination Group on Sustainable Cocoa Economy was initiated, our contributions within the topic of Pest and Disease Management were made easy. We had current facts and figures required, in hand, due to the very existence of INCOPED. This was through Country Reports from our Regional Coordinators presented in our past Seminars and documented in the proceedings. Further, the familiarity between us, stemming from previous seminars, branched out into frequent personal communications between members of our Group.

But, it is only fair that we admit that we have had a few downs, too. The circulation of the INCOPED NEWSLETTER was our pride, however we could not keep this up – for lack of contributions. In spite of repeated request for information and even going electronic, we were not successful. However, I take consolation that the reason for this is, as in the similar case of the International *Phytophthora* Group, due to the fact that most potential authors to the Newsletter gave more importance to publishing full papers in reputable journals, as this is a measure of professional standing.

Also, a further point that continues to worry me is that INCOPED has no fixed funding that can meet the cost of publications, invite speakers to our seminars, and have emergency meetings to deal with quarantine problems or pest and disease outbreaks in a cocoa producing country. If the latter situation were to arise, I cannot overemphasise, it would demand immediate action by highly qualified personnel to formulate an urgent strategy. This is an INCOPED function. And if I may drawn from personal experiences, this was the case with coffee berry disease in Kenya, coffee leaf rust in Brazil and witches' broom in Panama and Brazil. The 'be prepared' motto from our Boy Scout days, or our fire brigade services, applies. The running cost of INCOPED has been borne over the years by CEPLAC, Brazil, but with no continued guarantee. INCOPED is grateful to host countries that have supported the realisation of seminars. Today, our thanks go to the Cocoa Board of Ghana, and also Masterfoods that were kind enough to provide financial support.

After my marathon summary of the 14th ICRC, I will not go into the importance of insect pests and pathogens. Suffice to say: we chose to cultivate cocoa in the hot and humid environment preferred by the host-crop but likewise favoured by fungal pathogens; furthermore, away from natural enemies to pests and diseases encountered in the source of origin, and finally using as a base, a narrow cocoa genetic pool.

With that, Mr. Chairman, Ladies and Gentlemen, and with the ground set by our gracious INCOPED National Organisers, we can look forward to two satisfying days of work.

Thank you Ladies and Gentlemen – thank you all for being present.

Bertus Eskes

Chairman of the International Group for Genetic Improvement of Cocoa (INGENIC)

Dear Mr. Chairman, Prof. Yaw Ahenkora,
dear Mr. Kwame Sarpong, Chief Executive of the Ghana Cocoa Board,
dear Mr. Sona Ebai, Secretary General of COPAL,
dear Colleagues of the INCOPED Board,
dear Ladies and Gentlemen,

It is a pleasure to welcome you, firstly on behalf of the local organising committee of the INGENIC Workshop, chaired by Dr. Yaw Adu-Ampomah of the Cocoa Research Institute of Ghana, and also on behalf of the international INGENIC Committee that is co-organising this 4th INGENIC Workshop. We are pleased with the number of participants showing the wide interest that this event has generated. We are also very pleased with the joint arrangements made with INCOPED, which generates synergies and opportunities for the participants to attend sessions of both meetings.

INGENIC was created 10 years ago at the 11th ICRC held in Yamoussoukro, Côte d'Ivoire. The objective of our Group is to promote exchange of information and of ideas, as well as promoting collaborative efforts in the field of cocoa breeding and genetics.

The themes of the first three workshops were « Cocoa Breeding Strategies » in 1994 in Malaysia, « Contribution of Disease Resistance to Cocoa Variety Improvement » in 1996 in Brazil, and « New Technologies and Cocoa Breeding » in 2000 in Malaysia. These were themes of current interest in cocoa breeding and genetics. The choice of these reflects the underlying desire to promote discussions and synergies between different disciplines aiming at a common goal, which is the development of better cocoa varieties for farmers.

The theme of the current Workshop on « Cocoa Breeding for Improved Production Systems » is of great importance. Average productivity of cocoa is still very low and progress in improving efficiency is slower than in other tree crops. Low yielding varieties may be so because of low yield efficiency or because of poor adaptation to the environment. Low yields are sometimes obtained even under highly favourable growing conditions, which is an apparent paradox. Cocoa breeding may have overemphasised vegetative growth, which is important for rapid establishment of young trees, but which may be a disadvantage for adult plantations due to strong interplant competition and difficult management of the crop. Yield decline with age of the plantation is a common phenomenon. The continuous spread of devastating pathogens and pests also implies the need for lower cocoa canopies that can be more easily managed. These important challenges need to be faced by breeders and agronomists to help cocoa farmers.

These issues will arise in the topics that will be dealt with in our workshop:

- Efficiency of selection of mother trees,
- Factors affecting yield x vigour relationships,
- Needs and possibilities of developing compact cocoa varieties, and
- Genotype by environment interactions in cocoa, including high density planting.

We are very glad to have an invited speaker, Dr. Chris Atkinson of the Horticultural Research Institute at East Malling in the UK, to explain how dwarfing rootstocks were developed over the last century in temperate fruit tree crops. The objective of this work was to "obtain smaller and more easily manageable trees" which is an important aspect of our discussions on cocoa.

We are also glad to receive the active participation of cocoa physiologists and agronomists together with cocoa breeders and geneticists to discuss the traits that cocoa varieties need to possess to yield more efficiently and to be adapted to different ways of cocoa cultivation, with respect to the environment and to the needs of cocoa farmers.

INGENIC is grateful to the many institutions that provide general support to the functioning of our Group, as well as specifically to this Workshop. We wish to extend our special thanks to:

- The Cocobod, for sponsoring the joint cocktail and dinner,
- The CRIG for the organisation of this Workshop,
- The COPAL, for having this Workshop in conjunction with the 14th ICRC,
- The BCCCA and the World Cocoa Foundation, for financial support
- The "Stiftung der Deutschen Kakao- und Schokoladenwirtschaft", The Technical Centre of Agricultural Development (CTA) of the European Union, and the United States Department of Agriculture (USDA) for sponsoring several researchers and invited speakers, and
- Masterfoods, for sponsoring an invited speaker and providing additional supplies for the cocktail and dinner.

I thank you for your attention and wish you all interesting discussions and a fruitful Workshop.

WORKSHOP SYNTHESIS, CONCLUSIONS AND RECOMMENDATIONS

Topic 1. General Cocoa Breeding Aspects

Cocoa Production Systems

- Cocoa production systems vary greatly within and between countries. Most cocoa production is still based on low-input management. There are however tendencies to increase inputs (fertilizer, labour), to reduce overhead shade, to adopt pruning to limit tree height and canopy density, and to associate cocoa with other profitable trees (fruit trees, timber).
- Cocoa planting materials are still largely made up of unselected seedling populations. Selected hybrid varieties represent less than 30% of the world cocoa area. Often farmers rely on their own source of planting material. The use of cocoa clones is increasing in Asia and in America.
- Strong competition between cocoa trees is often observed in older cocoa plantations, which may be a factor in yield decline. Common cocoa varieties may be too vigorous, especially so under favourable growing conditions and at commonly used planting densities.
- Little information is available on the possible interaction of cocoa genotypes and production systems (shaded versus unshaded, high input versus low input cultivation, pruned versus unpruned, *etc.*).
- There is a tendency to involve farmers directly in the identification of promising genotypes and to carry out validation of new varieties in farmers' fields (*e.g.* presentation of Uilson Lopes, CEPLAC, Brazil). Such would help to ensure that new varieties are adapted to the farmers' growing conditions.

Cocoa Breeding Programmes

- Objectives of major cocoa breeding programmes include: yield potential, resistance to major diseases and pests, production uniformity and stability, easier and less costly management and improvement of quality characteristics.
- To be successful, a breeding programme should be comprehensive, balanced, with sufficient magnitude of scale. Continuity and sustainability are of utmost importance. These conditions are only rarely being fulfilled in any of the cocoa producing countries.
- There is an increased tendency to select clonal cocoa varieties rather than hybrid varieties. Clonal varieties allow for more rapid genetic progress that can be fixed in asexually propagated varieties. Selection of clones is to be considered even in Africa, where traditionally only seed varieties are grown.
- Growing of clonal cocoa varieties requires important changes in cocoa cultivation practices. Although adoption of clonal cocoa may be difficult under some conditions, availability of outstanding clonal cocoa varieties may be a stimulus for the farmers to improve production and management practices.
- Clonal cocoa varieties are mostly multiplied through budding or grafting. This poses the question of the possible effects of the rootstocks on the scion, which is still poorly understood with cocoa (see Topic 5).

Recommendations

- *Similarities between cocoa producing countries in the same region (America, Africa and Asia) justify regional approaches to cocoa breeding.*

- *Well supported regional centres or regional programmes would allow to obtain the critical mass of scientists and research activities required for success in cocoa breeding.A long-term commitment from donor agencies, the chocolate industry and producing countries is required to sustain cocoa breeding programmes.*
- *The workshop participants recommended that INGENIC set up a committee to propose regional breeding efforts and study the feasibility of establishing regional centres, regional programmes or regional projects.*

Topic 2: How to select superior mother trees in heterogeneous progenies for more productive clone or hybrid varieties

Causes and consequences of interplant variation in cocoa

- High correlated variation is observed in yield and vigour between trees in traditional cocoa hybrid trials. For example, in Ghana 17 to 27% of the trees were found to produce no useable pods and coefficient of variation (C.V.) between trees for pod yield varied from 30 to 76%.
- In PNG, high between-tree variation was observed for the number of flowers produced, for pollination efficiency and for the percentage of cherelle wilt. High yielding trees appeared to be able to bring a higher proportion of cherelles to maturity.
- In Côte d'Ivoire, variation for early yield between trees can be as high within clones as in hybrid progenies. This would suggest that the environment is a major factor inducing large tree-to-tree variations in yield. However, it was noted that in other datasets the tree-to-tree variation varied according to the genetic uniformity of the population (Lockwood, pers. comm.).
- Individual tree selection for yield in experimental plots, aiming at clone selection or at selection of mother trees for creating new seedling progenies, has so far been shown to be relatively inefficient.
- Visual observations in intensively managed clonal cocoa plantations would suggest that between-tree variation in yield can be reduced if uniform tree management is applied, including canopy pruning. However, more data analysis on this aspect would be required to draw firm conclusions.

Statistical approaches for improving individual tree selection

- Statistical methods that are helpful to increase the genetic value of the observations in heterogeneous stands are: "combined individual-family selection", "smoothing methods" derived from spatial statistics and "longitudinal data analyses".
- New experimental designs based on use of more than one individual per progeny plant will facilitate further increase in the genetic component of the variation observed. Such designs can be based on splitting the cocoa seeds into two parts ("true twins"), or on rapid vegetative multiplication of seedlings in the nursery as rooted cuttings, buddings or graftings ("accelerated hybrid clone selection").

Correlations between yield of individual trees in progeny trials and clones grown from them

- Correlations between early yield of individual trees and clonal progenies grown from them appeared to be low and non-significant in trials conducted in Côte d'Ivoire and in Malaysia.
- This is seemingly in contradiction with the relatively high heritabilities that have been reported for yield in some progeny trials.

- Other factors may therefore interfere when comparing yield performance of seedling trees with that of plagiotropic budded clones. It is important that these factors be better understood.

Correlations between mean yield of hybrid progenies and clones selected from them

- In Malaysia, ortet selection for yield efficiency was less effective than had been hoped, but nonetheless helpful. Family level selection for yield efficiency was more effective.
- In PNG, significant correlations were found between the family means and the yield of clones selected from these crosses in two sets of data, but not in another set of data.
- In Côte d'Ivoire, the average yield of mature hybrid progenies tended to be significantly correlated with that of the clones grown from individual trees from the same progenies, suggesting that family selection can be successful.

Correlations between yield of clones, observed in collections or in clone trials, and general combining ability for yield of the same clones used as parents in hybrid progeny trials

- The best set of data presented come from Malaysia (BAL plantations), where significant correlations were observed between yield of clones tested in trials and gca for the same trait.
- In PNG, also a good correlation was found between the yield of ten Trinitario parental clones and the average yield of the clones derived from progenies between the ten clones.
- Less conclusive results were presented from Côte d'Ivoire and Brazil, both for yield and yield efficiency. However, in Brazil positive correlations were observed for yield efficiency when comparing genetically different sets of clones in the CEPLAC collection, whereas genetically uniform clone sets produced non-significant correlations for the same trait.

Recommendations

- *More studies are needed to have a better understanding on the causes of tree-to-tree variation, comparing variation in seedling families with clones.*
- *Good experimental designs, sound agronomy and efficient data handling in breeding trials are fundamental to securing selection progress for yield.*
- *Ortet selection, based on family means, can be recommended to find better clones for yield and for yield efficiency. However, the correlation between family means and clones grown from them is not always significant.*
- *Ortet selection, based on individual tree yield alone, has generally not been reliable to predict the yield of clones grown from them.*
- *However, individual tree values may be useful to select for other, more heritable traits, such as disease resistance (as shown for black pod resistance in Côte d'Ivoire and for witches' broom resistance in Brazil) and quality.*
- *The use of rapid vegetative multiplication of seeds or seedlings ("true twin" and "accelerated hybrid clone selection") is recommended to speed up, and possibly increase, efficiency of ortet selection in new segregating populations.*
- *Individual clone values for yield, as obtained in clone trials, can be good predictors for yield of the hybrid progenies grown from these clones, as well as for the average value of the clones derived from these hybrids, provided that planting densities are near the optimum for the different clones. In other words, good clones are expected to produce good hybrids and good clones can be selected from those hybrids.*

- *Use of statistical methods may help to increase the knowledge on the genetic value of individual trees for quantitative traits, by introducing the family value and spatial variation parameters to correct individual tree values.*

- *More studies are however required to better understand the relationship between yield of seedling trees (or families) and plagiotropic clones grown from these trees (or families). These studies could involve the possible influence of plant growth habit (erect versus more horizontal plagiotropic branches, erect being favourable for clones whereas the growth habit seems less important for seedling trees) and of inter-plant competition (planting density, pruning intensity).*

Topic 3. Factors affecting yield x vigour relationships in cocoa

Scope and definition of yield efficiency (YE)

- Yield is considered to be the product of assimilate production and partitioning to the harvestable part of the trees.
- Efficiently producing trees have a favourable distribution of assimilates to the harvestable crop, in relation to total dry matter of the plant.
- Increases in production efficiency has allowed dramatic increases in yield per area associated with higher planting densities in a large number of crops.
- The high variability of partitioning biomass in cocoa offers an opportunity for the breeder, while taking into consideration the need to optimise planting density.
- In addition, higher production efficiency would reduce the need for pruning to keep the trees small (which is needed in cocoa to maintain profitable yields and to be able to manage the crop more easily).
- Total dry weight production of a tree is correlated with the trunk cross-sectional area (cm^2).
- Terms used to express biomass partitioning are "harvest efficiency", "yield efficiency", "cropping efficiency" or "harvest index".
- Hereafter, yield efficiency (YE) is defined for cocoa as dry bean weight divided by the increment (in cm^2) of the trunk sectional area measured at a uniform height above the soil over a certain period of time.
- Calculations of YE may either be based on dry bean yield and stem girth increment over a certain number of harvests, or on total yield and the final stem girth attained in the experiment.

Correction for vigour in cocoa breeding trials

- Large variation for tree size and vigour exist for cocoa genotypes (size of the adult tree canopy may vary 4-6 fold).
- Relative differences in vigour between small and large clones increases with time (PNG).
- Selection of clones based on yield alone will tend to favour the more vigorous clones, which may however not be adapted to the planting density in which they were selected.
- In breeding trials, yield values should therefore be corrected by the plant vigour, in order to evaluate the YE of the genotypes, and to include smaller genotypes in the selection process.
- Depending on the genotype, optimal planting densities for clones may vary seven-fold (700 to 5000 trees per ha), as has been demonstrated at BAL Plantations in Malaysia. Optimal densities depend also on general growing conditions, such as soil fertility and management (*e.g.* PNG).

- In selection or validation trials, cocoa varieties with known differences in vigour level should be planted at different planting densities. Such is carried out routinely for clone trials in PNG.

Genetic and environmental factors affecting YE

- Large additive components of genetic variance have been observed for YE as well as for yield and vegetative vigour (Malaysia).
- Although yield and YE (as defined above) are generally quite closely correlated, the best progenitors for yield are not necessarily the best progenitors for YE and the best yielding clones may not have the highest YE.
- In PNG, YE is more responsive to increase in planting density than yield.
- Furthermore, YE decreased after three cropping years in PNG for small as well as for big clones. This is because stem girth increased with time, but yield reached a peak after three cropping seasons. This behaviour appears to be quite common in fruit crops.
- In PNG, smaller clones at higher densities appear to have, in average, a better yield and a better YE than bigger clones planted at lower densities.
- However, it is not well known if it is a general trend that YE decreases with increase in tree vigour. In fact, evidence exists that big genotypes can also be rather efficient (e.g. BR25 clone).
- Little is known so far about other environmental and agronomic factors (pruning) that may affect YE.

Physiological traits related to efficiency of cocoa trees

- Total dry matter production is the product of photosynthetic capacity and quantity of solar irradiation intercepted by the canopy, which are affected by the leaf area index and the canopy architecture.
- Higher photosynthetic capacity may not necessarily lead to higher productivity, as other factors may interfere, such as partitioning of assimilates between vegetative and generative parts.
- More open canopies would favour light distribution in the canopy. Such canopies have lower light extinction coefficients.
- Exploitation of variation for canopy architecture and tree vigour in breeding would allow for selection of genotypes adapted to high-density plantings that may be highly productive.
- Genotypes with a relatively high photosynthetic rate under low light intensity that could be better adapted to yield well under high shade conditions might be found.

Effects of inter-tree competition

- A general competition effect between neighbouring trees is observed in cocoa stands planted at normal densities. This effect has been observed quite early (*e.g.* 18 months at density of 1667 trees per ha), and may then not be due to vegetative vigour, but to other causes such as attractiveness to insect pests.
- Inter-plant competition, generally attributed to an imbalance between vigour and planting density, is increasing with time and may be partly responsible for "yield decline", as observed in ageing plantations in many countries.
- Inter-plant competition may disappear after drastic reduction of the planting density (thinning). Thinning of 50% of the trees may drastically increase individual tree yield to the extent of maintaining or even increasing the same yield per ha as before thinning (*e.g.* Côte d'Ivoire, French Guiana).
- Under the conditions of the French Guiana trial reported, cocoa families could be classified as "aggressive", "stimulating", or "passive" in relation to their neighbours.

- Interplant competition effects of families are explained best by variations in the trunk section and not by the yield or by the yield components. Hence, it should be possible to select non-aggressive high yielding families.

Recommendations

- *Aiming at obtaining more efficient cocoa trees, YE is a trait that should be taken into account in any breeding programme.*
- *It is therefore recommended that measures of stem girth be taken routinely in all breeding trials and even in collections, in order to be able to calculate YE over certain periods of time.*
- *It is recommended that studies be conducted to obtain measurements of YE that are less correlated with yield itself. Such would be useful to obtain a variable that carries more weight in selecting for highly efficient cocoa trees.*
- *Official recommendations for commercial cocoa varieties should be done for groups of hybrid progenies or of clones exhibiting similar vigour levels and growth habit, and for which the optimum planting density has been identified.*
- *Genetic and plant husbandry (pruning, planting density) pathways need to be explored further to decrease competition effects between neighbouring trees and optimise YE.*

Topic 4. Possibilities to use rootstocks for developing more efficient and compact cocoa trees

General

- The development of more compact cocoa trees is desirable to increase yield and YE as well as to facilitate tree management (pruning, harvesting, spraying).
- Compact cocoa trees may be developed through selection of less vegetatively vigorous trees and possibly also through the use of "dwarfing rootstocks".

Lessons learnt with temperate fruit trees

- The development of dwarfing rootstocks in temperate fruit tree cultivation is the single most important factor increasing YE by reducing scion varieties size and allowing for high density planting.
- Dry matter partitioned to fruit in dwarfing *Malus* (apple) rootstock can be 70%, compared to 40-50% in more "vigourating" rootstock varieties.
- Positive effects of selected rootstocks include also increased precocity, fruit size and quality, tolerance to climatic stress and pest and disease resistance (including resistance to *Phytophthora* sp).
- The rootstocks scion dwarfing effect on growth is independent of cropping, it is not a simple change in yield efficiency. Most dwarfing rootstock x scion combinations have smaller amounts of roots than less dwarfing combinations.
- The vigour of the rootstock when grown on its own is not always related to the degree of dwarfing induced by the rootstock. Some "dwarfing rootstocks" can be quite vigorous when grown on their own roots, and not all low-vigour genotypes have dwarfing effects when used as rootstocks.
- Dwarfing effects of low-vigour rootstocks can be enhanced by grafting higher up the rootstock stem.
- Dwarfing effects can also be obtained by using inter-stem grafting.

- Mechanisms of dwarfing rootstocks are not yet fully understood. However, hormonal factors, variation in nutrient and water movement, and partial obstruction at the graft union have all been suggested to be involved.
- Dwarfing rootstocks in apple generally appear to have a high phloem-xylem ratio. This trait can possibly be used as a selection criterion to find new dwarfing rootstocks also in other fruit crops.
- Alternative approaches to induce dwarfing effects include use of growth regulators, restriction of root growth and of irrigation at critical times.

Rootstock experiences in cocoa

- Rootstocks are used in a wide range of crops to compensate for deficiencies in otherwise excellent scions, such as difficulty in rooting and long juvenile periods. Very often these scions have qualities that are highly valued in the market. These considerations do not apply to bulk cocoa.
- However, there may be interest in cocoa to try to increase yield efficiency, allowing for high density plantings, and to obtain resistance to root disease by selecting suitable rootstock varieties.
- Rootstocks for commercial cocoa clones are generally seedlings obtained by open-pollination. In some American countries, rootstock varieties with resistance to *Ceratocystis fimbriata* have been selected and used.
- Most relevant yield data using seedling rootstock varieties of cocoa come from three trials carried out in Malaysia. In two trials there was no main effect of hand-pollinated or open-pollinated rootstock varieties. In another larger trial, there was significant variation in early yield and vigour but not in YE among twelve hand-pollinated rootstock varieties, but interaction with three scion varieties was generally absent. The SCA 6 x SCA 12 rootstock produced the largest scions and also the highest yield, but below average YE.
- Only one experiment has been reported with clonal rootstock varieties (Murray and Cope, 1959, Report on Cacao Research, Imp Coll. Trop. Agr. Trinidad, pp.29-35). The authors of this paper concluded that there was no prospect of producing higher yields with particular combinations than with single clone plants, that it is possible to increase the yield of a weak clone like ICS45 by growing it on a vigorous stock, and that yields on a "mutant" dwarf were extremely poor.
- However, as pointed out during the workshop by some participants, the results presented in the above paper might be interpreted differently when analysed from the point of yield efficiency. The rootstocks had significant effects on YE (with ICS1 having 50% lower YE than ICS60) and the highest yield and YE were obtained when grafting the best scion (ICS1) onto the best rootstock (ICS60). The least vigorous rootstock clone (ICS45) produced the lowest yields but not the lowest YE. This may suggest that yield can be further enhanced by choosing appropriate densities for rootstock/scion combinations giving high YE.

Prospects of using mutant seed varieties as rootstock in cocoa

- In PNG, a mutant genotype MJ12-226 was found among progenies of the SCA 12 x NA 149 cross. It has small and narrow leaves, a smaller root system and strong branching habit, suggesting weak apical dominance. The mutation appears dominant with possible interactions with the cytoplasm.
- Mutant seedlings when used as rootstocks produced a dwarfing effect on the scions in the nursery. This effect persists at least till one year after field planting. No data are yet available on older grafted plants.
- The MJ 12-226 mutant trait is also of potential value as a scion or seedling, interesting for its multi-stem, orthotropic growth habit.

- In Ghana, the potential of the crinkle leaf mutant (CLM) as a possible source for dwarfing rootstock is being investigated. CLM is characterized by small crinkled leaves, short internodes, a smaller root system and smaller pods and beans. CLM appears to be dominant trait, with a 2:1 ratio being observed in hand pollinations when using CLM as female parent.
- Initial field studies (not yet conclusive) show that CLM has potential in reducing the shoot growth of the scions.

Recommendations

- *In the search for compact cocoa varieties, there are favourable prospects to select for more efficient and compact scion varieties. In the discussions, the urgent need for developing such better scion varieties was generally stressed.*
- *Opinions varied on the prospects and need for developing "dwarfing" rootstocks for cocoa. Some participants considered that this is not a priority, because the advantages expected from dwarfing rootstocks can possibly be developed also in good scions, irrespective of the rootstock.*
- *Other participants considered however that developing good rootstock varieties (dwarfing and with other important traits) is also important, because of increasing interest in commercial use of clonal varieties, that are generally multiplied by grafting or budding. High vigour of otherwise good commercial clones (e.g. PBC123 and CCN51) might be corrected by adequate rootstocks.*
- *Rootstocks with uniform positive effects on the scions would be more interesting than rootstocks that cause important interactions in different combinations.*
- *Rootstocks in cocoa have still been poorly studied; more understanding is required on how "dwarfing rootstocks" can be developed in cocoa.*
- *Research experience related to rootstock in apple may possibly help to speed-up the development of good rootstock varieties in cocoa. For example, studies on phloem/xylem ratio and on grafting at different heights of low-vigour rootstock seedlings could be tried.*
- *Care is to be taken with grafting of vigorous scions onto low-vigour rootstock. If budding or grafting is done too low (e.g. hypocotyls-type of budding or grafting), the scion may develop its own roots and the rootstock effect may be lost (experience in French Guiana).*
- *Crosses with related species in the same genus deserves retrying using a wide range of combinations for introgression of important traits, including rootstock effects.*

Topic 5. Analysis of genotype by environment (GxE) interactions in cocoa

Stability of cocoa varieties over sites

- Information presented on other occasions suggested relative small interactions between hybrid cocoa varieties and sites between cocoa growing regions in the same country (*e.g.* Brazil, Côte d'Ivoire and Ghana).
- Multilocational trials at two sites in PNG failed to show significant interactions between clones and sites.
- However, strong interactions of clones and sites were observed in a recent trial in Indonesia. These interactions might however be partially explained by different plant husbandry standards applied at the sites, with high experimental errors observed at one of the two sites. For some clones the

interaction with sites appeared to be related to differential susceptibility to VSD, *Helopeltis* and *Phytophthora*. Four clones showed stable and good yields over both sites.

- Early results on recent multilocational clone trials in Brazil suggest larger GxE interactions for yield than for witches' broom resistance. The use of 10 trees per clone and per site results in similar selection progress as 20 trees per clone for both traits.
- Soil factors appeared to be more important than geographic distance and climate factors on GxE interactions for early yield.
- Ongoing research (International Clone Trial in the CFC/ICCO/IPGRI project) is expected to throw more light within the next five years on the stability of traits of cocoa clones over ten different countries.
- Little is known yet about the magnitude of interactions of cocoa genotypes with production systems (low against high input, shaded versus unshaded) and pruning regimes.

GxE interactions for planting density

- As indicated above, earlier results have shown a wide variation for the optimal planting density for cocoa clones.
- Such has been clearly demonstrated in Trinidad in the early 1990's for three TSH clones planted at three densities (748, 1495 and 2990 trees per ha). One clone was specifically well adapted to the high planting densities (TSH919).
- In Ecuador, the EET19 clone also showed best adaptation to higher planting densities (1000 compared to 666 and 500 trees/ha).
- Recent results from Indonesia and PNG showed little interaction between clones and planting density. This could possibly be ascribed to the relatively low variation in planting densities used in these trials (625 and 1000 trees per ha in PNG and 800 to 1333 trees per ha in Indonesia).

High density commercial planting

- Good yields (1.5 to 2 tonnes per ha) can be sustained over many years by high density planting of TSH clones or of TSH seedling progenies in Trinidad. The high inputs required are considered as economically advantageous in relation to "improved low density planting".
- Adoption rates of high density planting by smallholders in Trinidad have however been low.

Recommendations

- *Further studies into the interaction of genotypes and environment are required to match planting materials with local growing conditions and plant husbandry regimes (e.g. planting density, pruning).*
- *Promising new clones need to be tested over a wide range of environments before general recommendations can be made on the range of adaptation of individual clones.*
- *Clones can be found with good adaptation to many sites, however the possibility of selecting clones adapted to specific environments should not be excluded.*
- *High density planting can be economically advantageous in cocoa, provided adequate plant husbandry practices are applied, including intensive pruning.*

Topic 6. Other perspectives related to development and multiplication of new planting materials

Farmer-researcher participatory on-farm selection of new varieties

- Integration of knowledge of farmers and of breeders in selection of new varieties has shown to be successful in Brazil, dealing with an emergency situation aiming to find good witches' broom resistant varieties.
- Results of an on-farm survey on planting materials in Nigeria show implications for participatory breeding and for adoption of research results by farmers. Farmers tend to give priority to trees that yield well throughout the entire year and that are relatively less vigorous. The ideal tree was considered to possess a mixture of traits found in Amelonado and in Upper Amazon cocoa types.
- Nigerian farmers were further able to identify trees with reduced incidence of black pod.

Prospects for rapid multiplication of scion and clonal rootstock varieties for cocoa

- Results in Trinidad and Malaysia have shown similar yield potential for clones that are grafted onto seedling rootstock using plagiotropic or orthotropic budwood.
- Use of plagiotropic grafted clones appeared superior to use of plagiotropic rooted cuttings for adult tree yield in one trial in Ecuador. Further studies would be useful on this subject.
- Commercial production of orthotropic plantlets directly through somatic embryogenesis is viewed as being very expensive. This must be incorporated with downstream nursery multiplication to improve feasibility when utilized for germplasm transfer or other purposes
- Bending of orthotropic shoots in the greenhouse or in a budwood garden has been used with success on large scale, be it experimentally, for rapid multiplication of orthotropic budwood or grafts. Simple heading pruning of low-forming jorquettes can be used to avoid canopies forming at different, and too low, heights.

Recommendations

- *Use of farmers' knowledge on planting materials and direct involvement of farmers in the selection process are important new approaches in the development of better yielding and more resistant cocoa varieties. Such approaches are expected to provide higher adoption rates of selected varieties by farmers.*
- *Comparison between orthotropic versus plagiotropic rooted cuttings should be considered, if clonal dwarfing rootstocks are to be developed.*
- *Rapid multiplication of orthotropic cuttings, budwood or graftwood is probably best obtained by bending orthotropic shoots in budwood gardens.*

On-Farm Selection for Witches' Broom Resistance in Bahia, Brazil – a Historical Retrospective

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Abstract *

Witches´ broom, caused by *Crinipellis perniciosa*, was introduced in Bahia in 1989, causing severe economical, social and ecological losses. Since the beginning, resistance was chosen as one of the key points of the integrated pest management adopted by the Cacao Research Center (CEPEC). Unfortunately, by the time WB arrived in Bahia, around 600 thousand hectares were planted as a continuous monoculture, mostly with susceptible varieties. Fortunately, among the varieties deployed to farms in the past, some resistant parents were included and possibilities of finding resistant segregant plants in a large screening program were expected. Today, ten years after the beginning of the program, more than one thousand trees were pre-selected by CEPEC and more than five thousands pre-selected by farmers. Among these, highly productive and resistant clones have been found. In this paper, we report the strategies adopted, the results and some drawbacks of that program.

** This is the summary of the invited paper presented by the first author during the joint opening session of the INGENIC and INCOPED workshops. The full paper will be published as part of the proceedings of the 14th International Cocoa Research Conference, where this paper was presented as a poster.*

Breeding Strategies to Improve Cocoa Production in Papua New Guinea

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"The disturbance to our operations is due to that GxE garden; the cocoa production from the research block is so eye catching that villagers want to have some direct benefit from the sale of the wet beans." Stephen Mombi, Extension Officer, East Sepik.

Abstract

It is generally agreed that very little progress has been achieved in cocoa breeding to date. Too high an emphasis on breeding for disease resistance, heterozygosity of hybrids' parental clones and insufficient use of proven breeding methods were described as some of the possible reasons for the poor progress. To be successful, a breeding programme should be comprehensive, balanced and of sufficient magnitude of scale. Continuity and sustainability of the breeding activities are of utmost importance. Those involved with the cocoa breeding programme in Papua New Guinea have attempted to follow these general guidelines since 1994. The objectives, strategies and activities undertaken by the Cocoa and Coconut Research Institute are described in this paper under the headings of *parental clones*, *hybrids, hybrid clones* and *multi-location testing*. As hybrid clones are unfamiliar to cocoa farmers in Papua New Guinea, representing a new production system, particular emphasis was placed on adaptive research and preparation of extension materials in anticipation for the release to the farmers of the first varieties of hybrid clones in early 2003. The establishment of three regional centres for cocoa research is proposed to ensure the availability of sufficient resources, sustainability and continuity that are required to achieve adequate progress in cocoa breeding.

Introduction

There is more than adequate genetic variability in cocoa for breeders to achieve vast improvement in yield (Warren and Kennedy (1991). Yet it is generally agreed that very little progress has been achieved in cocoa breeding to date. Hunter (1990) stated that for the Western Hemisphere "outside of few varieties, most of which have not been subjected to vigorous testing, little is currently available for farmers in the way of superior planting materials." Lockwood (2003) argued that "cocoa breeders have not delivered what today farmers require". Too high an emphasis on breeding for disease resistance, which often became the only objective and an end in itself with very little attention given to yield improvement, was given by Kennedy *et al.* (1987) as the main reason for the slow progress in cocoa breeding. Commercial planting of cocoa hybrids, derived from two heterozygous parental clones that show a high degree of genetic diversity and phenotypic variation, was also mentioned as a reason by Warren and Kennedy (1991). Lockwood (2003) concluded that "proven breeding methods have not been used enough".

Cocoa is a tropical tree crop grown mostly in the developing countries. Being a cash crop, it was not included in the mandate of the International Centres for Agricultural Research System (CGIAR) that concentrate only on food crops. National programmes had to rely mainly on scanty local resources or *ad hoc*, sporadic, short term and un-coordinated bilateral arrangements with donors.

To be successful, a breeding programme should be comprehensive, balanced and of sufficient magnitude of scale. It has to be done in a logical sequence whereby each step of the breeding work leads to the next step until the final goal of releasing improved planting materials to farmers is achieved. Moreover, continuity and sustainability of the breeding programme are of utmost importance. Starting from 1994, the Cocoa and Coconut Institute (CCI, formerly CCRI) of Papua New Guinea (PNG) has attempted to follow these general guidelines to develop a comprehensive cocoa breeding programme. The purpose of this paper is to describe briefly the breeding objectives and strategies and the activities undertaken to achieve them at CCI as an introduction for the fourth INGENIC International Workshop on '*Cocoa Breeding for Improved Production Systems*'.

Background

Papua New Guinea (PNG) is usually blessed with good cocoa growing conditions, that is with good soils, adequate rainfall sufficiently spread throughout the year without a very long dry period and almost optimal temperatures. Under these conditions, production can start as early as 18 months after planting. However, these conditions also promote vigorous vegetative growth.

Cocoa was first introduced into PNG from Samoa by the Germans at the beginning of the 20th century. The germplasm was Trinitario that originated from Trinidad and Venezuela reaching Samoa via Java, Ceylon (Sri Lanka) and Cameroon. Upper Amazonian germplasm was introduced in the 1960s by the then Department of Agriculture, Stock and Fisheries (DASF).

Initially, all the cocoa was grown on large estates, predominantly in association with coconut. In some areas, cocoa was grown under leguminous shade trees. Then, in the early 1950s, smallholders began planting cocoa in small plots. At present, more than two-thirds of the annual crop is produced by smallholders. The majority of them copied the plantation system, either by being employed as labourers, or through extension organised by various settlement schemes. Thus, the major production system in PNG is a plantation style system of cocoa grown in monoculture either under coconut palms or *Gliricidia sipium* shade. The size and level of management of cocoa blocks vary from one farm to another.

Most of the cocoa germplasm used in PNG until the 1980s was Trinitario, usually derived from seeds of open-pollinated pods. Some farmers are still using the same practice. In the 1960s, a very severe epidemic of Vascular Streak Dieback (VSD) disease caused by the fungus *Oncobasidium theobromae*, destroyed many plantations in PNG. Breeding efforts were initiated with a major emphasis to develop VSD resistant planting materials. Surviving Trinitario trees were cloned, 13 of which were released as planting material with an acceptable level of resistance to VSD. Commercially, however, the released clones were grown in only a few plantations. Their main use was as parents to develop hybrids crossed with locally selected Upper Amazonian clones (KEE series) developed from seeds introduced in the 1960s from Trinidad.

The first hybrid, SG1, was released in 1982. The major selection criteria were yield and VSD resistance. At that time, *Phytophthora* pod rot (Ppr) was not considered a major constraint for cocoa production in PNG. Thus, several clones with high susceptibility to Ppr (KA 2-101, KA 5-201, K 24-102) were included as parents of the SG1 hybrid. Consequently, Ppr became more widespread and destructive. It was therefore necessary to develop the SG2 hybrid, using different, more Ppr resistant clones as parents. The SG2 hybrid was released in 1988, originally as a poly-cross hybrid composed of 15 different crosses.

The SG hybrids were more precocious and higher yielding than the previously planted Trinitario materials. Experimental results promised high cocoa yields. This,

combined with high cocoa prices at the time, led to a high adoption rate of the hybrids, which was supported by the Agricultural Development Bank and other agencies. As a result, most of the cocoa presently grown in PNG is either the SG1 or SG2 hybrids. Unfortunately, as high as the expectations were, the disappointments were even higher.

In 1994, the Cocoa and Coconut Research Institute (CCRI, now CCI) modified its breeding strategy to emphasise mainly, but not only, the development of clonal planting materials. A change in breeding objectives and strategies was required. Then, later in September 1994, all the existing cocoa breeding trials had to be terminated due to severe damage caused by a volcanic eruption. New breeding activities and trials were initiated in 1995 according to the Section's new objectives, taking advantage as much as possible of the germplasm and data obtained in previous years.

An overview

The major aim of the CCI's breeding programme is to develop new cocoa varieties that will improve the profitability of cocoa farms. Attempts are being made to:

- Increase yield potential;
- Improve production uniformity and stability;
- Increase resistance levels to major diseases;
- Reduce management costs;
- Improve quality characteristics;
- Develop ecologically targeted varieties.

Emphasis is being placed on the development of hybrid-derived clones (hybrid clones) with higher yield potential and better uniformity as compared to cocoa hybrids. However, the programme also continues to develop and test new cocoa hybrids. Both types are for release as ecologically targeted poly-cross hybrids or poly-clonal varieties to minimise the risk of genetic vulnerability. The hybrid clones are targeted at advanced growers whilst the hybrids have been developed for use in remote areas and by less advanced growers. Particular emphasis is being given to potential vigour to minimise interplant competition and to optimise recommended agronomic practices , particularly planting density.

The hybrid vigour expressed in crosses between the Trinitario and Upper Amazonian germplasm is the major source for hybridisation and cloning. Attempts are also being made to explore the combining ability within the Upper Amazonian and the Trinitario populations. It is recognised that the performance of newly developed hybrids or hybrid clones greatly depends on the quality of the parents used. As such, special considerations are being given to widen and improve the genetic base of the two populations. This is being done by introduction, collection of local germplasm (Trinitario) and population improvement.

The major breeding activities are being carried out at CCI's headquarters at Tavilo, East New Britain Province. Currently, there are 28 different established activities (trials) on an area of about 125 ha. Additional breeding activities are carried out at Stewart Research Station (SRS), Madang Province on an area of about 30 ha, with major emphasis on screening for VSD resistance. Several multi-location trials (GxE) were also established in cooperation with the Cocoa and Coconut Extension Agency (CCEA) and several large-scale commercial plantations.

The various activities are divided into five projects:

- Introduction, development and selection of improved parental clones
- Development and testing of new experimental hybrids
- Development and testing of new hybrid clones
- Multi-location testing of advanced hybrids and hybrid clones
- Special projects (factors affecting yield variability, rootstock x scion interaction, sunlight tolerance, orthotropic buddings, *etc.*).

Three senior staff, a Section Head (PhD.) and two Research Officers (MSc.) are in charge of the programme with the assistance of eight Assistant Research Officers (Diploma in Agriculture) and 82 labourers. Some of the activities are supported by the CFC/ICCO/IPGRI International Cocoa Project and Masterfood International. However, most of the activities are financed from CCI's internal resources derived to a large extent as income generated from the cocoa breeding trials. The Cocoa Breeding Section is cooperating closely with other Sections of CCI.

Parental clones

Cocoa is an allogamous species and heterozygous by nature. Cocoa breeding, similar to other cross-pollinated crops is largely based on the phenomenon of heterosis or hybrid vigour. Thus, the identification of suitable heterotic groups should be the foundation of any cocoa breeding programme. At CCI, the locally adapted Trinitario and the Upper Amazonian germplasm are being used as the two main heterotic groups. These two groups are genetically variable. The breeding challenge is to identify or develop within each one of them the best possible genotypes (clones) to provide progenies that fulfill the specific requirements of the breeding programme. These genotypes should:

- Have good combining ability for yield with the reciprocal population;
- Be good donors for resistance to disease and insect pests;
- Be good donors for desirable vigour and plant type;
- Provide desirable pod, bean and quality characteristics.

Three different activities are included under this category at CCI:

- Introduction;
- Collection of locally adapted germplasm;
- Population improvement.

Introduction of new germplasm

Introduction, if successful, is the cheapest and fastest "breeding programme". Unfortunately, good final products (hybrid or clones) are not readily available. Therefore, we have to depend on "public" germplasm that is available in intermediary quarantine stations. However, these materials can be very useful as parents or as donors for specific attributes. The most significant introduction into PNG was that of the Upper Amazonian germplasm from Trinidad in the 1960s. It was introduced as seeds of crosses between related clones, for example, NA 33 x NA 34 or SCA 6 x SCA 9, from which the KEE clones were developed. The KEE clones are widely used, as such, in the breeding programme.

Seventy-four clones were introduced more recently from either The University of Reading or CIRAD, Montpellier, 27 of them through the CFC/ICCO/IPGRI Project. The clones were established in a germplasm collection block. Thirty-one of these clones are included in the CFC International Clone Trial (ICT). Several clones were preliminarily identified as promising for future potential use in the breeding programme as follows: AMAZ 15-15, EET 308, IMC 85, IMC 105, MAN 15-2,

POUND 7, PA 107, PA 150, SIAL 339 and T85/599. However, additional information is required.

The value of introduced clones as parents was observed in the CFC Local Clone Trial (LCT). Several high yielding hybrid clones, having an introduced clone in their pedigree (AK), were identified (Table 1). The LCT is an observation trial of one replication. The value of these clones should be verified in an advanced replicated trial.

Table 1. Yield, yield components, vigour and reaction to *Phytophthora* pod rot (Ppr) of several experimental AK and control hybrid clones observed during 2000-2002

Clone	Pedigree	Pod wt (g)	Wet bean (%)	Total dry bean (kg/ha)	Ppr	Vigour
AK 56-1-4	KEE 43 x SIAL 93	952	25.1	5942	5.3	B
AK 57-1-9	KEE 43 x POUND 5C	666	31.8	5901	2.0	I
AK 54-2-6	KEE 43 x UF 11	860	28.5	5199	1.3	S
AK 76-2-16	KEE 48 x SIAL 93	346	32.5	5174	2.5	S
16-2/3 (C)	KEE 42 x K82	601	29.0	4715	1.2	I
73-14/1 (C)	KEE 12 x K24-102	378	34.2	4709	7.5	I
37-13/1 (C)	KEE 43 x KA2-106	785	27.4	4058	5.2	B

B = Big I = Intermediate S = Small C = Control

Collection of local Trinitario germplasm

Since the beginning of the 20th century, cocoa production in PNG was based on Trinitario germplasm. It was propagated by seeds from open-pollinated pods harvested probably from selected productive trees. Since Trinitario germplasm is typically cross-pollinated and heterozygous, highly variable Trinitario germplasm evolved. This germplasm was exposed for a long time to various constraints including poor management in some cases. Surviving, good yielding and healthy trees should be very useful, locally adapted genetic materials for the breeding programme. Unfortunately, this germplasm is gradually disappearing as a result of replanting with the SG hybrids or because the trees are becoming too old.

Moreover, it was realised that the breeding programme is based on a very narrow Trinitario genetic base, mainly because of susceptibility to Ppr and VSD, the two most common diseases. The SG2 hybrid was originally based on three Trinitario clones as parents – KA2-101, KA 2-106 and K 82, all developed from VSD surviving trees. The crosses with KA 2-101 were later removed because they were too susceptible to Ppr. From the remaining two clones, K 82 is relatively susceptible to VSD and KA 2-106 is susceptible to Fish Bone Disorder (Efron *et al.* 2002e). It was essential, therefore, to widen the genetic base of the Trinitario germplasm.

The Trinitario germplasm collection was initiated, in 1995, in various parts of the country. Both, large and small-scale cocoa blocks were visited. Preference was given to older trees and the recommendations made by the farmers. Budwood was collected from about 400 trees across the country and the clones developed were established in an observation trial. The results were surprisingly very good and encouraging. Few of these clones were included in the replicated CFC, ICT (Table 2). Some of these clones ('Old' Trinitarios or "OT") were as high-yielding as the

released hybrid clones, and significantly higher yielding than K 82, the Trinitario control. They also showed good levels of resistance to Ppr and VSD.

Collection and preservation of germplasm is an important activity, but it will be fruitless as long as it is not being used either directly or in the breeding programme. Selected OT clones have already been planted in an advanced yield trial. In addition, experimental hybrids in crosses with Upper Amazonian testers were developed and are presently being tested. Based on the initial pod production, some of the hybrids are promising. Selected OT clones were included also in crosses made recently for the second cycle of population improvement of the Trinitario germplasm.

Table 2. Dry bean yield and other variables of 'Old' Trinitario (OT) and control hybrid clones in the Local Clone Trial (CFC project) between June 2001 and April 2003

Clone	Type	Total yield (kg/ha)	Pod wt (g)	Wet bean %	Annual yield efficiency (kg/cm2)	Ppr (0-9)	VSD * (1-6)
K 72-46/51	OT	2438	398	28	18.0	1.9	2.4
36-3/1 (C)	TA	2423	765	28	13.5	1.6	3.7
NAB 11	OT	2344	768	27	14.3	2.3	2.1
T 11	OT	2148	497	42	9.0	4.1	2.5
K 72-153/4	OT	2063	393	25	12.5	3.4	2.4
K 78-3	OT	2026	395	29	14.6	1.9	2.3
17-3/1 (C)	TA	1979	446	30	11.8	2.5	2.3
K 82 (C)	T	914	396	28	7.2	2.4	3.4

OT = Old Trinitario, T = Trinitario, TA = Trinitario x Upper Amazonian , and C = Control
* Results from SRS, Madang

Population Improvement

Today, at the beginning of the 21st century, cocoa breeding is still based, to a large extent, on clones from populations such as Scavina, Nanay and IMC, which were collected by Pound from the wild more than 50 years ago. Valuable as these materials were and still are, not much further progress can be expected if these clones are being used as parents repeatedly in breeding programmes. Each one of these clones has positive attributes, but some undesirable characteristics as well. Experience has shown that most of the economically important variables in cocoa are inherited polygenically and additively. The purpose of population improvement is to combine the desirable genes into a small number of genotypes. Two Trinitario clones, K 82 and KA 2-101, can provide a simple example. Both clones produce hybrids of similar yield in crosses with the KEE Upper Amazonian clones. However, K 82 is a good donor for Ppr resistance but a poor one for VSD. Conversely, K A2-101 is resistant to VSD, but highly susceptible to Ppr. Is it not possible to select among the progenies of a cross between these two clones genotypes that will be resistant to both Ppr and VSD?

Taking the above into consideration, in 1995 CCI initiated a population improvement project for the Trinitario and Upper Amazonian germplasm. Advantage was taken of two sets of within population diallel crosses of the Trinitario and Upper Amazonian germplasm. Individual progeny trees were selected for cloning based on data collected and visual observations made prior to the volcanic eruption. Higher yielding clones, by a magnitude of 50-100% relative to the controls, were identified in

both populations (Table 3). Clones from the Trinitario population were recently test-crossed with four Upper Amazonian testers. At the same time, crosses between Trinitario clones, including selected 'Old' Trinitario clones were also made to initiate the second cycle of population improvement. A similar crossing programme is planned for the Upper Amazonian germplasm in 2004.

Population improvement is a long term or even permanent project. Significant progress can be realised probably only after several cycles. However, some progress can be achieved following each cycle, whereby selected clones can be used as parents to produce new experimental hybrids and hybrid clones. It is a demanding project, but it should start and continue in a sustainable manner in order to achieve future progress in cocoa breeding.

Table 3. Average annual dry bean yield and yield components of selected Trinitario x Trinitario derived clones in comparison with three control Trinitario clones

Clone	Pedigree	Pod wt (g)	Wet bean (%)	Dry bean (kg/ha)	Yield efficiency (%)
T39-141	KA2-106 x K23	418	31.7	1926	220
T710-143	K6 x 58/24	328	44.3	1810	206
T610-2-5	K20 X 58/24	396	47.3	1458	166
T56-1-11	KT140 x K20	474	25.3	1378	157
Rum Jun 2 (c)	Unknown	323	29.6	877	100
KA2-101 (c)	Unknown	370	28.9	580	66
K82 (c)	Unknown	411	25.7	502	57

c = Controls

Cocoa hybrids

Two cocoa hybrids, SG1 and SG2, were developed and released in PNG. The SG1 hybrid was released in 1982 as a VSD resistant hybrid mixture. However, it was susceptible to Ppr. The second hybrid mixture, SG2, was released in 1988 as poly-cross hybrid of 15 different crosses. It was more resistant to Ppr, but less resistant to VSD. Farmers identified several problems related to the two types of hybrids:

- High production variability between trees;
- Early yield decline;
- Vigorous growth that requires frequent pruning;
- Insufficient resistance level to Ppr.

Modification of the SG2 hybrid

The SG2 hybrid was developed based on results obtained from a progeny trial (P.T 101) of Trinitario x Upper Amazonian clones. Data from this trial were available for nine years from 1984 to 1992. The complaints of the farmers were verified (Figure 1) since the total number of pods during this period ranged from less than 200 to more than 1600 pods per tree. The average coefficient of variation (CV) within SG2 crosses was high, ranging from 45.4% in 1984 to 102.1% in 1992 (Figure 2). The yield reduction phenomenon was observed starting from 1987 (4^{th} year of production) and, in 1992, the yield was 40.3% of that in 1986 (Figure 2). The yield reduction was associated with an increase in the CV for the number of pods within crosses. Natural

infection by Ppr in the 15 crosses ranged from 6.5% to 31.3%, and their relative vigour index ranged from 60 to 139.

Based on the above analysis it was realised that due to the heterozygosity of the parental clones, the inherent tree-to-tree variability in production cannot be avoided. The reasons for early yield reduction were not understood and therefore no means to an immediate improvement could be visualised. However, it was envisaged that improvement could be achieved by dividing the SG2 hybrid into two components, SG2-B (big) and SG2-S (small), based on the relative vigour of the trees, and by eliminating the crosses with KA 2-101, which were the most susceptible to Ppr. This was done, and each component was composed of five crosses in variable proportions based on their calculated value index and the need to maintain, as far as possible, a balance of the different Trinitario and Upper Amazonian parents within each one of the two SG2 components (Table 4). Modifications and improvements in the methodology of seed production and distribution were also proposed.

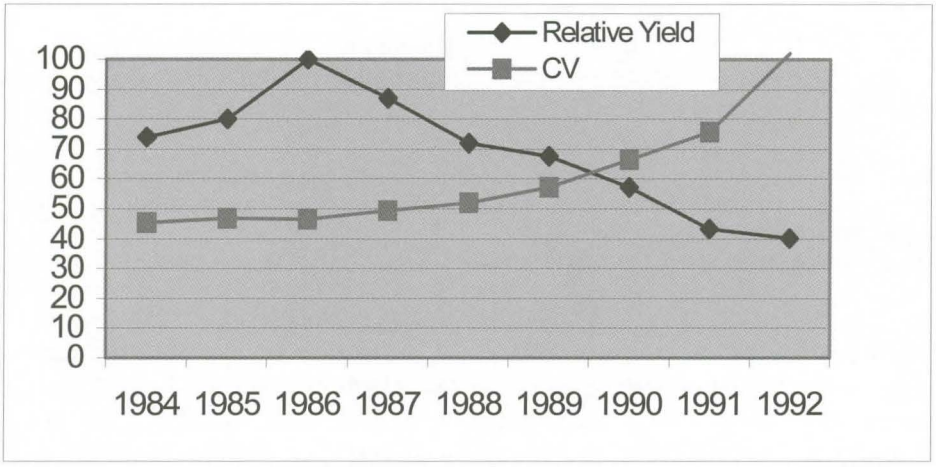

Figure 1. Frequency distribution (%) of total number of pods/tree in progenies of two SG2 crosses during 1984-1992

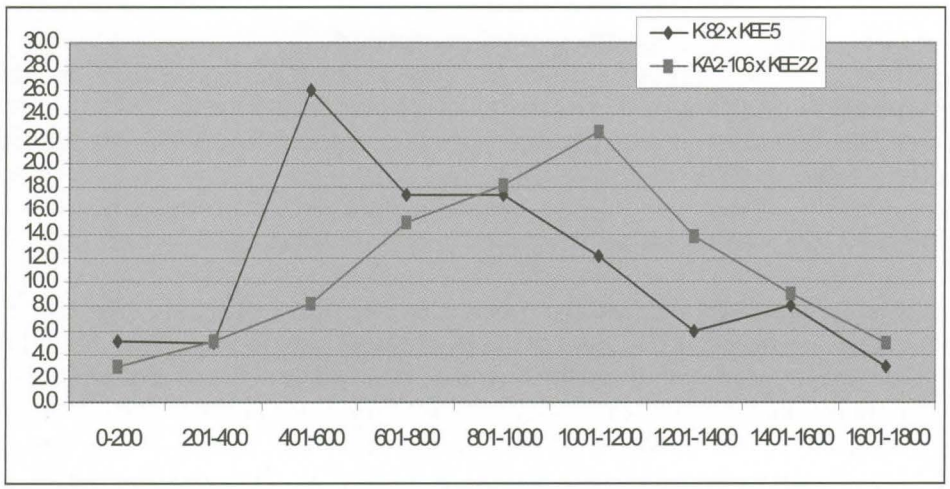

Figure 2. Relative yield (%) and average coefficient of variation (CV) within the 15 original SG2 crosses during 1984 - 1992

Table 4. Composition of the SG2-B and SG2-S hybrids

SG2-S					SG2-B				
Cross	Value[1] Index	Rela-tive[2] vigour	Ppr[3] (%)	Propor-tion[4] (%)	Cross	Value[1] Index	Relative[2] vigour	Ppr[3] (%)	Proportion[4] (%)
KEE 5 x K 82	40	73	9.9	20	KEE 42 x K82	51	108	10.8	40
KEE 12 x K 82	52	60	6.5	25	KEE 5 x KA2-106	29	112	22.7	10
KEE 43 x K 82	29	70	12.5	10	KEE 42 x KA2-106	53	111	16.5	20
KEE 12 x KA 2-106	61	80	11.9	25	KEE 43 x KA2-106	51	129	13.5	20
KEE 23 x KA 2-106	41	86	26.7	20	KEE 47 x KA2-106	34	127	21.1	10

1) Value index calculated based on yield, percent Ppr and quality characteristics
2) Relative vigour based on trunk circumference, tree height and canopy width
3) Proportion of Ppr infected pods
4) Proportion of seeds within the hybrid

Factors affecting yield variability and yield reduction in the SG2 hybrid

Tree to tree variability in production and yield reduction were the two major problems encountered with the SG2 hybrid. A study aimed at understanding the reasons for yield variability and reduction was initiated in cooperation with researchers from other disciplines. Forty trees from each one of the SG2 crosses were planted and the number of flowers, cherelles and pods per tree were counted for two years. Later, the experiment was modified to test more thoroughly only 16 trees per cross. Results from this study are presented and discussed in a separate paper in these Proceedings (Efron *et al.* 2003a).

Can yield variability be reduced by partial inbreeding?

The Trinitario clone, K 82, is one of the parents of the SG2-B and SG2-S hybrids. It is self-compatible and one of the most resistant clones to Ppr in PNG. Considering that one cycle of self-pollination reduces heterozygosity by 50%, an attempt was initiated to develop more homozygous clones from K82 by one cycle of inbreeding (S1). Selected K 82-S1 clones may have the potential to produce a more uniform hybrid than K 82. Identification of clones more resistant to Ppr can be an added benefit.

Seventy-five trees derived from self-pollination of K 82 were cloned. Thirteen of them were rejected because of poor growth, probably due to inbreeding depression. The remaining clones were planted in the field in1998. The heterozygous nature of K82 was clearly evident from the results of the clones derived from it (Table 5). Among others, the variability was expressed in the size, shape, surface characteristics and colour of the pods (Figure 3). Six selected K 82-S1 and K 82 were test-crossed with four Upper Amazonian testers (parents of the SG2 hybrid). Progenies of these crosses were planted in the field recently. Results from this experiment can provide an answer as to whether better uniformity can be achieved by developing partially homozygous parental clones.

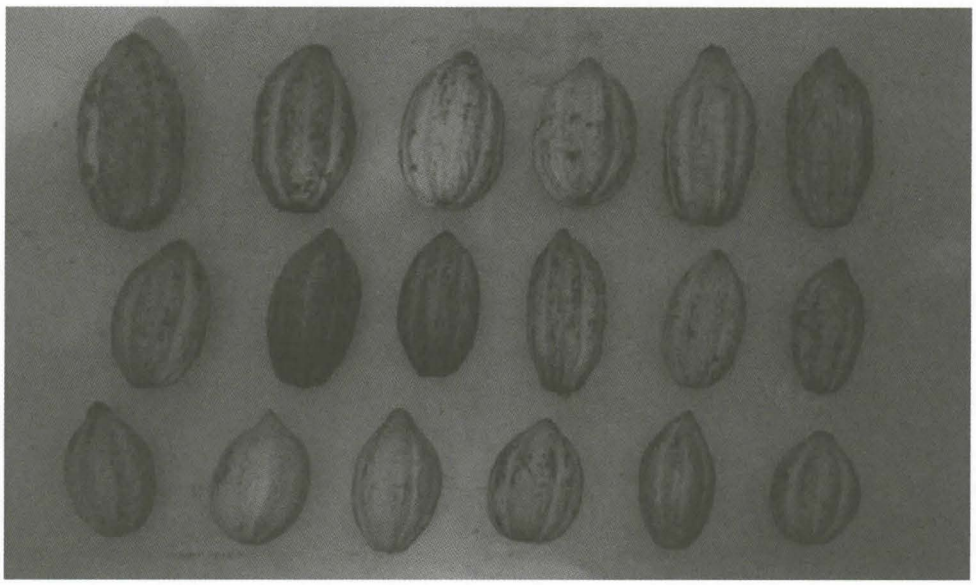

Figure 3. Pods of K82-Selfed

Table 5. Average yield, yield components and reaction to *Phytophthora* pod inoculation of 10 selected K82 S1 clones

Clone	Pod wt (g)	Wet bean (%)	Dry bean (kg/ha)	Ppr (1-9)
K82/52	700	17.1	1509	3.1 R
K82/22	341	36.0	1263	6.6 S
K82/18	592	28.8	1261	8.5 HS
K82/19	404	32.2	1149	2.9 R
K82/7	720	20.4	1125	2.0 HR
K82/74	540	31.5	1097	6.8 S
K82/21	421	27.8	1091	3.0 R
K82/71	424	29.8	1037	3.9 R
K82/67	393	23.0	999	7.9 HS
K82/28	465	23.1	971	1.8 HR
KA2-101 (c)	376	27.4	722	7.3 HS
K82 (c)	443	27.4	415	2.5 HR

C = Control; HR = Highly resistant; R = Resistant; S = Susceptible; HS = Highly susceptible

New Experimental Hybrids

Development of new hybrids is an objective of the cocoa breeding programme. New experimental hybrids are also being used as a source to select new hybrid clones. Several hybrid trials were established since 1995 as follows:

2.4.1 Hybrids between selected Trinitario x Upper Amazonian clones. These were double-cross type hybrids produced mainly to identify productive mother trees for cloning. Clones developed from this trial are being tested presently in an

observation trial. In addition, a Ppr resistant cross was identified. Seeds were distributed to several plantations for semi-commercial testing.

2.4.2 Hybrids produced at BAL Plantation, Malaysia, mainly between Upper Amazonian clones. It was intended that these be included in a GxE study involving both Malaysia and PNG. However, it was not tested in Malaysia due to the sale of BAL Plantation. These hybrids did not produce higher yields than the SG2 control crosses. Most of them had small beans of less than 1.0 g (Efron *et al.* 2002d).

2.4.3 Hybrids between selected "Old" Trinitario clones and Upper Amazonian testers. Production in this trial has started recently. Some of the hybrids look promising.

2.4.4 Hybrids between new Trinitario clones and Upper Amazonian testers derived from the first cycle of population improvement. The trial was planted only recently.

Hybrid clones

Most of the commercial tree crops grown in the world are clones. Cocoa production, in contrast, is based mainly on hybrids, and this is the case in PNG. Considering the heterozygousity of the cocoa trees, the high tree-to-tree variability in production and that cocoa can be propagated vegetatively, it is only logical to select and clone the highest yielding genotypes of the hybrid population and to use them for commercial production. Breeding considerations, strategies and methodologies were adjusted accordingly. The term "hybrid clone" is used to indicate that they derived from hybrid trees and to distinguish them from the pure Trinitario or Upper Amazonian clones that are being used as parents.

Trees of the same clone are by definition genetically identical, each producing potentially the same yield under the same environmental conditions. However, genetic uniformity can be an obstacle to progress in breeding by making the plantings more vulnerable to diseases and insect pests. Therefore, CCI has adopted a policy of releasing only poly-clonal varieties whereby the variety is composed of several clones, each of different pedigree. At the same time, the clones within the variety should have similar vigour to minimise interplant competition and to ensure that agronomic recommendations, particularly planting density, are equally applicable to all of the clones.

Adaptive research and extension associated with hybrid clones

Farmers in PNG are familiar with cocoa hybrids propagated directly from seeds. Clones are propagated and grown differently. They require different management practices, particularly during the first year after planting. Therefore, the commercial production of hybrid clones is a new cocoa production system, unfamiliar to most farmers in PNG. Simultaneous adaptive research, training of extension personnel and preparation of written materials were essential in anticipation of the release of the hybrid clones.

Propagation

Cocoa can be propagated vegetatively by either cuttings or various budding techniques. Past experience in PNG showed that cocoa trees derived from cuttings tend to lodge more easily due to the absence of a tap root. CCI already had good experience with the patch budding technique. This was combined successfully with the technique developed in Malaysia to bud two week-old rootstock (juvenile

budding). A special bulletin "*A Guide to Juvenile Budding*" was prepared and training courses for extension personnel were organised.

Rootstock
The effect of the rootstock on scion growth and productivity is not well understood. A comparison between rootstock obtained from hybrids of different vigour did not show any effect of the rootstock on the growth and productivity of the scions used. Accordingly, large beans of commercial cocoa are being used.
More recently, a growth mutant with a dwarfing effect as rootstock was identified at CCI (Figure 4). Research with this mutant is in progress. Results obtained so far are presented in these Proceedings (Efron *et al.* 2003b).

Figure 4. Mutant rootstock

Budwood gardens
A well-established source of budwood is required before clones can be released to growers. Preferably, it should be in the vicinity of nurseries. Awareness of the need to establish budwood gardens was introduced together with recommendations for management with a particular emphasis on adequate labelling. Budwood gardens were established in cooperation with the Cocoa and Coconut Extension Agency (CCEA) in several provinces.

Formation pruning
Cocoa growers in PNG are familiar with hybrids that are propagated by seeds. They grow initially as single-stemmed plants that later "jorquette" to develop five primary fan branches. Clones are propagated by budding onto rootstock using plagiotropic (fan-branch) buds. Budded plants may not grow straight, and usually follow the lateral growth habit of fan branches. Formation pruning, a skill that does not exist as yet in PNG, is required to direct the buddings to grow upright in a balanced way.

Adaptive research was conducted to develop simple guidelines for shaping the budded trees. Tipping about six months after planting to remove apical

dominance and promote development of side branches followed by removal of very low or undesirable branches six months later were identified as the main features of formation pruning. A bulletin "Formation Pruning of Cocoa Clones" and a simple leaflet "*How to Get More Pods from your Hybrid Clones*" were prepared.

Orthotropic buddings

Budding can also be done using orthotropic buds (Efron *et al.* 2000c). The resulting trees grow like the hybrids with single upright growing stems. Formation pruning is not required. Therefore, it is expected that, if orthotropic buddings can be done on a commercial scale, the newly developed hybrid clones would be more easily accepted by cocoa growers, particularly in the small scale sector.

Several problems associated with orthotropic buddings were identified and investigated:

a) Initial source of orthotropic budwood

A very low frequency of spontaneous orthotropic growth from plagiotropic buddings was observed in some genotypes. However, the CCI Breeding Section's research has shown that orthotropic growth can be induced by heavy pruning at the level of secondary branches of mature trees (Table 6).

Table 6. Frequency (%) of orthotropic growing shoots in heavily pruned clones

Clone	Type	No. shoots/tree	Orthotropic shoots (%)
K82	Trinitario	19.3	10.9
KA2-101	Trinitario	14.1	38.9
KEE 43	Upper Amazonian	69.0	22.2

b) Availability of orthotropic buds

A special orthotropic budwood garden is required in order to obtain a large number of orthotropic buds for commercial production. The recommendations developed include planting the orthotropic buddings at a high density of 5000 trees per hectare, growing them for one year to develop sufficiently strong stems and pruning all the jorquette branches except one to induce growth of orthotropic shoots from the main stem. Thereafter, the trees are maintained in the orthotropic state by cutting the upper part for budwood.

c) Jorquette height

Orthotropic buddings grow like hybrid seedlings. However, they jorquette much lower (Figure 5). CCI's research has shown that jorquette height depends on several factors such as the source of budwood (primary or secondary orthotropic branches), the genotype, the age of the rootstock and shade conditions. It was also found that jorquette height could be tailored to the desirable height by tipping the growing point.

Figure 5. Orthotropic budding: jorquette height

d) Uniformity of sprouting
The success rate of orthotropic budding is usually as good as that of plagiotropic budding. However, orthotropic buds tend to be dormant resulting in late and non-uniform sprouting. Therefore bud sprouting is not uniform and takes longer than plagiotropic buds. Improvement can be achieved by pre-tipping the growing point 1-2 weeks before collection of budwood. Notching above the budding point also improves sprouting. However, the rate of sprouting is still an obstacle for large-scale production of orthotropic buddings. Additional research is required.

Orthotropic buddings obtained during the research were distributed or sold to farmers. They were received enthusiastically by them. Some farmers are paying well in advance, waiting for more orthotropic buddings to be produced by the breeding programme.

The use of hybrid clones
Hybrid clones, like hybrids, are used to establish new plantations, but they can also be used to replace/rehabilitate existing plantations. However, farmers in PNG and probably elsewhere are reluctant to cut trees in preparation for planting. A technique was developed to rehabilitate existing hybrid cocoa trees. The technique is based on induction of chupon growth by pruning, field patch budding of selected hybrid clones on the chupons and gradual removal of the older trees. A bulletin "*A Manual for Cocoa Rehabilitation by Chupon Budding*" was prepared and training courses for extension personnel were conducted.

Vigour and planting density
The potential vigour of a cocoa tree is under genetic control. Trees of different sizes can be developed by breeding. However, each size-group may have a different optimal planting density. It should be tested in order to formulate the necessary recommendations for farmers. For example, three groups of big, intermediate and small sized clones, with 11 clones per group, were tested at two densities of 625 and 1000 trees per hectare. The relative vigour of the trees was confirmed by measuring

trunk circumference (Table 7). The small clones responded better than the other two groups in terms of the total yield produced in the first five years and in terms of the annual yield efficiency calculated as dry beans produced per cm^2 of the stem cross sectional area (Table 8). Additional details are presented in these Proceedings (Efron *et al.* 2003c).

Table 7. Average trunk circumference of small, intermediate and big clones at two densities at Tavilo, seven years after planting

Clone size	Trunk circumference (cm)		
	Low density	High density	Average
Big	43.5 A (A)	39.6 A (B)	41.6 A
Intermediate	38.5 B (A)	35.3 B (B)	36.9 B
Small	36.4 C (A)	34.3 B (B)	35.3 C
Average	39.5 (A)	36.9 (B)	

Numbers showing the same letters are not statistically significant at the 5% level (Newman-Keul's test). The letters in brackets refer to the density effect within the size groups

Table 8. Average trunk cross-sectional area, total yield and yield efficiency of three size groups of clones planted at 625 and 1000 trees per hectare

	Big clones			Medium clones			Small clones		
	H	L	H/L	H	L	H/L	H	L	H/L
Cross sectional area (cm^2)	124.8	150.6	0.83	99.2	118.0	0.84	93.6	108.4	0.86
Total yield (kg/ha)	7545	6837	1.10	7656	6908	1.11	8468	6775	1.25
Yield efficiency (kg/cm^2)	12.1	9.1	1.33	15.4	11.7	1.32	18.1	12.5	1.45

H = High density (1000 trees/ha) L = low density (625 trees/ha)

Hybrid clones – breeding considerations

Logically, the best breeding approach to develop elite hybrid clones is to select the highest yielding, disease resistant progeny trees of the best available hybrids. This was the initial approach taken at CCI. Thus, the identification of heterotic groups and parental clones with good GCA within each group, followed by the identification of pairs of parental clones with high SCA are of fundamental importance to cocoa breeding. Unfortunately, we are still in the dark, and whatever knowledge we have is empirical, based on trial and error. Experience in CCI has shown that some parental clones provide higher proportions of high yielding hybrid clones than other parental clones and that various degrees of correlations were obtained between yield of the hybrids and the proportion of high yielding hybrid clones derived (Efron *et al.* 2003d).

Accelerated hybrid clones breeding scheme

Two problems were encountered with the breeding of hybrid clones. Assuming new crosses are being produced and the hybrids are being tested initially in a progeny trial, the breeding cycle to develop hybrid clones would take more than 20 years. In addition, the experience in PNG and elsewhere has shown that usually there is a poor correlation between the performance of mother trees and the clones derived from them. Taking the above into consideration, CCI has developed and is implementing an accelerated hybrid clones breeding scheme that can shorten the breeding cycle by 8-10 years (Efron *et al.* 2002b). The scheme is based on the following outlines:

Activity	**Duration**
1. Crosses between selected parents	6 months
2. Nursery planting, screening for Ppr by the leaf disc test and negative selection of 25-30%	4 months
3. Planting at high density (10,000 plants/ha) in a VSD 'sick plot'. Select resistant plants	12 months
4. Nursery juvenile budding (2 week-old seedlings)	4 months
5. Field planting of large numbers of clones at high density (1,667 plants/ha.) Evaluation of the clones for precocity, yield potential and other characteristics. Observation trial of 4 trees/clone.	4 years
6. Advanced trial of selected clones	8 years
Total	**14 years**

The nature of the scheme requires a large number of genotypes at the start. At CCI, not having previous knowledge of the families used, the scheme started with about 6000 genotypes from 29 families developed at BAL Plantation, Malaysia (Efron *et al.* 2002b). Following the leaf disc test for Ppr, approximately 4000 genotypes were cloned and planted in the field. Only 6.3% of them were selected for advanced testing at the end of year 4. The selected clones were recently planted in an advanced replicated trial.

The relationships between mother trees and derived clones

Experience has shown that the correlation between mother trees and clones derived from them is poor. This experience is based mainly on observation rather than solid experimental data. In the absence of better criteria, clones are still being selected based on the performance of mother trees. However, due to the poor correlation, more mother trees should be selected to increase the probability of identifying elite, desirable clones.

A mother tree is an individual single genotype in a segregating population whose performance is greatly influenced by the environment. Is it possible to improve the precision of selecting mother trees for cloning by replicating their genotype several times? An experiment supported by the CFC/ICCO/IPGRI "*Cocoa Germplasm*

Utilization and Conservation, a Global Approach" was established to address this question. A total of 480 genotypes, 20 each from 24 different hybrid families, were replicated by orthotropic budding using budwood from the original seedlings. After regrowth, the original seedlings were planted in a completely randomised design in the field as replicate number one (Rep 1). Three additional budded seedlings from the same genotypes were planted in replication 2, 3 and 4. About one year later, when both chupons and fan-branches were available on the trees in Rep 1, 48 trees (2/family) were randomly selected for cloning by orthotropic and plagiotropic budding.

The 48 clones were planted in six replications, three of orthotropic and three of plagiotropic type budding, with four trees per replication. The following information for various variables was expected from the experiment:

- Correlations between the original seedlings in Rep 1 and the th?ree budded seedlings derived from them in reps 2-4.
- Correlations between the randomly selected 48 clones and the original seedlings (Rep 1) as compared with the correlations with the average performance in the four replications.
- Comparison between the performance of orthotropic and plagiotropic budded seedlings and the relative correlation of the two groups with the performance of the original mother trees.

Preliminary results showed various levels of within family correlations between the original progenies and the average of the three budded trees derived from them (Table 9). Thus, all the correlation coefficients in the cross K 4 x KEE 12 were highly significant whilst only the jorquette height was significantly correlated in the cross K 6 x KEE 12. Different levels of correlations were obtained for the various variables measured. Dry bean yield, pod weight and trunk circumference were less frequently significantly correlated. Most of the correlation coefficients for jorquette height, percentage of wet bean and yield efficiency were significant. Overall correlation coefficients, using data from all crosses, were significant and varied between 0.32 and 0.69. The highest coefficient was that for yield efficiency, showing that this trait might be more correlated within family than yield or vigour.

Disease resistance

Diseases are major constraints to cocoa production worldwide. Resistant varieties provide the easiest, most cost effective, environmentally friendly solution to farmers. Therefore, breeding for resistance to major diseases should be an important component of any breeding programme.

Phytophthora pod rot (Ppr) and Vascular Streak Dieback (VSD) are the two major diseases in PNG. There is more than adequate genetic variability available for resistance. However, there is no point to having a variety or clone highly resistant to one disease, but susceptible to another. Combining yield potential and disease resistance is even more important.

The development of appropriate and reliable methodology to screen large numbers of genotypes, preferably at an early age, is a prerequisite to a successful resistant breeding programme. The methodology should be as simple and inexpensive as possible. The leaf disc test for Ppr could have been an ideal screening methodology, as its results were proven to correlate with field data in other countries. Unfortunately, so far, it has not worked satisfactorily in PNG. Meanwhile, the detached pod spray inoculation test is being used. The results of this test are relatively well correlated with field observations. The main limitations are the time required to obtain pods and the number of pods that can be tested at any given time.

A promising simple and inexpensive methodology was recently developed for VSD screening, taking advantage of the relatively high disease pressure at the Stewart Research Station, Madang (Efron *et al.* 2002a). It consists of planting the

test materials in an established "sick" plot at a very high density of about 10,000 plants ha^{-1} for a period of 12 to14 months. During this period, the plants are constantly exposed to a high level of natural infection by VSD. New test materials are inter-planted during wet periods about 3-4 months before the removal of the previously tested materials and thus immediately exposed to a uniform infection by VSD. The plants are assessed periodically, and those that show mild symptoms for several months are selected as resistant genotypes. This method enables us to effectively test large numbers of genotypes at low cost in a relatively short period of time, in a small area.

Table 9. Correlation coefficients (r) of various variables measured from the original progenies and three budded primary orthotropic trees between January 2001 and March 2003

	Variables					
Cross	**Dry bean yield (kg/ha)**	**Pod wt.(g)**	**Wet bean (%)**	**Trunk circum. (cm)**	**Y. E**	**Jorquette height (cm)**
K4 x 82	0.79 **	0.49*	1.0**	0.45*	0.39	0.72**
K4 x KEE 12	0.57 **	0.85**	0.55**	0.65**	0.79**	0.63**
K4 x KEE 43	0.05	0.49*	0.79**	0.49*	0.52*	0.64**
K4 x 16-2/3	0.05	0.62**	0.72**	0.28	0.74**	0.86**
K4 x 17-14/4	0.92 **	0.55*	0.41	0.80**	0.94**	0.73**
K4 x 36-3/1	0.47 *	0.21	0.59**	0.70**	0.83**	0.74**
K4 x 38-10/3	0.52 *	0.51*	0.56*	0.50*	0.90**	0.63**
K6 x K82	0.42	0.53*	0.62**	0.44	0.24	0.26
K6 x KEE 12	0.35	0.12	0.39	0.27	0.12	0.51**
K6 x KEE 43	0.68 **	0.57**	0.29	0.62**	0.81**	0.82**
K6 x 16-2/3	0.35	0.43	0.76**	0.66**	0.54*	0.76**
K6 x 17-14/4	0.53 *	0.62**	0.79**	0.18	0.72**	0.79**
K6 x 36-3/1	0.71 **	0.90**	0.71**	0.20	0.88**	0.19
K6 x 38-10/3	0.27	0.39	0.90**	0.60**	0.83**	0.48*
K9 x K82	0.11	0.10	0.98**	0.12	0.71**	0.66**
K9 x KEE 12	0.26	0.65**	0.58**	0.62**	0.29	0.69**
K9 x KEE 43	-0.48 *	0.62**	0.66**	0.45*	0.48*	0.48*
K9 x 16-2/3	0.04	0.18	0.45*	0.089	0.32	0.47*
K9 x 17-14/4	0.20	0.07	0.41	0.15	0.85**	0.71**
K9 x 36-3/1	0.47 *	0.43	0.49*	0.11	0.74**	0.74**
K9 x 38-10/3	0.61 **	0.38	0.22	0.43	0.73**	0.67**
K82 x KEE 12	0.04	0.19	0.91**	0.73**	0.66**	0.42
K82 x KEE 43	0.43	0.76**	0.18	0.73**	0.71**	0.63**
KA2-106 x KEE 12	0.20	0.11	0.72**	0.52*	0.58**	0.29
Overall	0.32**	0.57**	0.56**	0.50**	0.69**	0.38**

*, ** = Significant and highly significant at 5% and 1% levels of significance, respectively
Y.E = Yield efficiency

Testing of hybrid clones

Currently, there are eight different experiments aimed at testing and selecting new promising hybrid clones. Some of them are duplicated at SRS, Madang. These

experiments are composed of germplasm from various sources. The testing is being done either in advanced replicated trials or as observation trials with only one replication.

Multi-location testing

PNG is a diverse country with different environments reflecting various soil types, rainfall (amount and distribution), solar radiation, and prevalence of diseases and insect pests. Planting material, especially the genetically uniform clones, may respond differently to these environments and this would be reflected by significant GxE interactions. Therefore it is necessary to test the performance of elite planting materials at various locations representing the major cocoa growing ecologies.

The first multi-location testing of hybrid clones was initiated in 1995. Twenty-nine clones were included, divided into three plant size categories; big, intermediate and small. Each size group was planted in a separate sub-trial at two densities (625 and 1,000 trees ha^{-1}). Two previously released Trinitario clones K 82 and KA 2-101 were used as common controls. Significant differences were found between clones for yield, yield components and reaction to Ppr and VSD. Significant differences were found also between locations and planting densities. However, there were no significant genotype x environment interactions. The yields of several selected hybrid clones were twice as great as that of K 82, the highest yielding Trinitario control clone. Based on the results obtained, two poly-clonal varieties HC1-B with four big hybrid clones and HC1-S of four small hybrid clones were officially released by CCI in March 2003.

A second multi-location trial with 44 new hybrid clones was initiated in 2001. The trial is divided into four size groups, big, intermediate, small and very small, each planted at different densities of 625, 714, 833, 1000 trees ha^{-1}, respectively.

Sustainable cocoa breeding

Cocoa is a tropical tree grown in developing countries. Improved planting material, if properly developed by breeding, is the easiest technology for the farmers to adopt. It is the most effective way to increase cocoa production and improve the livelihood of the many farmers involved in cocoa production. It is relatively easy for farmers to adopt the new planting materials since cocoa is a cash crop rather than a traditional food crop. However, the developing countries lack the necessary resources to support adequate research and therefore national cocoa breeding programmes suffer from a chronic lack of resources.

Sporadically, a new star is shining in the sky, usually through the participation of a donor agency. Research activities are being initiated and some progress is being achieved. Unfortunately, these stars are not shining for long. It is not fully appreciated that breeding efforts, particularly in perennial crops, require long-term research. Moreover, the donor community tends to follow a fashion, and the projects submitted to them for support follow suit. However, as the fashion changes frequently, so do the projects and the support given to them. As a result, cocoa breeding research is far from being sustainable. No wonder that the progress achieved so far in cocoa breeding is not exciting. It may be of interest to compare it with the resources, the amount of research and the progress achieved, for example, in maize breeding in the U.S.A or Europe.

Cocoa breeding in PNG is based on two major premises, comprehensiveness and continuity. All the breeding elements starting from introduction and collection of germplasm to multi-location testing of elite materials and release of new varieties are included. Adaptive research, with or without cooperation of researchers in other

disciplines is also included. Moreover, it is also realised that every activity should have a purpose and continuity. If germplasm is being collected, it should be evaluated, and selected materials have to be used in the breeding programme as parents to produce new experimental hybrids or hybrid clones. Similarly, when an observation trial is being conducted, the next step must be an advanced replicated trial. It is believed that good progress has been achieved in PNG during the last ten years. Only time will tell how sustainable the efforts made are going to be.

However, it may not be necessary to have a similar magnitude of breeding activities in every cocoa producing country. There are many similarities between neighbouring cocoa producing countries. Resources can be pulled together to establish well-supported regional centres for cocoa research with the required critical mass of scientists and research activities. Long-term commitment from donor agencies, the chocolate industry and the producing countries is required. If done properly, significant progress can be achieved. Probably, it is the only way to achieve progress.

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How to Improve the Efficiency of Individual Cocoa Tree Selection for Quantitative Traits in Progeny Trials?

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Abstract

With perennial crops, it is necessary to select individual trees when applying certain selection strategies. Choosing individuals and multiplying them by vegetative propagation is an essential step in selection of new clone varieties. The selection of new parental genotypes within segregating progenies is a basic feature of recurrent selection schemes. For both these objectives, it is necessary to have reliable methods for choosing individuals in experimental plots. Various methods are proposed here for improving the selection efficiency for quantitative traits of individuals in segregating progenies in cocoa variety trials. Firstly, statistical methods are presented that make it possible to fine-tune the choice of trees for selection: "combined individual-family" selection, "smoothing methods" derived from spatial statistics and "longitudinal data analysis". Secondly, methods are proposed to improve experimental designs by increasing the number of trees per genotype: use of "true twins" and rapid vegetative propagation of individual seedlings. These different methods are discussed and ways of making the most effective choice of cocoa trees in experimental plots are proposed.

Introduction

The main purpose of experimental designs in selection trials is usually to control environmental effects, thus reducing error variance in analyses of variance and improving precision when comparing different varieties in trials. In cocoa trials, the planting material is usually made up of several full-sib families. The different families can be genetically independent from each other, in which case we talk about single-pair trials, or they may be linked to each other by sharing certain parents. Indeed, several mating designs make it possible to link full-sib families, such as "hierarchical" mating designs, "NC-II" factorial designs or "diallels" (Cilas 1991). These different crossing systems provide access to the genetic parameters ("general" and "specific combining abilities", "heritabilities", coefficients of "genetic correlation" between traits, *etc.*), needed to effectively select parents or families for further usage or dissemination.

Choosing individuals from progenies planted in field trials is needed either for the rapid creation of new varieties, in the form of clones, or for recurrent selection schemes to choose new parents from the progenies produced. However, success of individual tree selection depends essentially on the degree of heritability of the trait observed (calculated by using individual tree observations). If such heritability is low (0 to 0.2) then little success can be expected from individual tree selection , if medium (0.2 to 0.4) then some success can be expected and if high (above 0.4) then selection can be expected to be more successful. Heritability will largely vary with the degree of precision with which the trait can be observed. For example, broad sense heritability for individual tree yield has been shown to be nearly zero based on data for one year with low yield, up to 0.4 for a normal production year and as high as 0.6 when cumulative yield over several years is considered (Cilas *et al.* 1999).

Heritabilities can therefore be increased by more accurate observations, by correcting for observed environmental effects, or by multiplication of the number of replicates per genotype (for more accurate estimation of the genotypic variance against the environmental variance). The genetic parameters used for progeny selection may also enable a more accurate prediction of the genetic value of individuals in trials. In fact, such predictions use individual phenotypic values, and the phenotypic values of related individuals (Baradat *et al.* 1995). The rationale and possibilities provided by these methods are outlined hereafter.

Genotype uniqueness problems in progeny trials

As the cocoa tree is primarily a cross-fertilising plant, substantial genetic diversity exists within a family of full-sibs. When variety trials are set up in appropriate statistical designs, it is possible to classify the different families into groups of significance for observed traits. However, selection of individual trees in seedling progenies, aiming at clone selection or for use in new crosses, is not easy. The reason is that each individual derived from a cross is unique; there is no replication of the genotype. Therefore, no statistically significant differences between individual trees in trials can be calculated by conventional tests. Indeed, confusion can occur between the genetic value of the tree and the effect of its micro-environment. There are several ways of compensating for this drawback.

The uniqueness of genotypes in progeny trial plots calls for elaborate statistical procedures to reduce the risks of error in the selection process. There are two types of risk:

- selecting individuals with low genetic values, if for example the micro-environmental situation of those individuals is more favourable than average, and
- not choosing individuals with high genetic values, if for example they are in unfavourable micro-environments that mask their potential.

Before describing the statistical methods capable of fine-tuning individual tree selection in progeny trials, it is worth taking a look at the experimental designs used in cocoa variety trials.

Frequently used experimental designs

Choosing an experimental design means defining the size and shape of elementary plots and their distribution in field trials.

Different studies conducted to determine the most appropriate experimental designs for cocoa selection have usually led to recommendations for experimental designs consisting of totally randomised single-tree plots (Lotodé and Lachenaud 1988; Cilas 1995). The aim of these authors has always been to compare hybrid families.

From a practical point of view, designs with single-tree plots have a certain number of advantages and disadvantages.

Advantages are:

- The analysis can focus on living trees only (analysis of variance with unequal numbers), thus reducing any problems with missing trees or plots. On the other hand, in designs with multiple-tree plots, the plot means do not have the same precision when trees are lost, as they are then calculated using different tree numbers.
- Distribution of the trees of the different crosses on areas of the site with mediocre fertility is random in single-tree plot designs. However, in multiple-tree plot

designs, some families may be penalised more than others if some of the experimental plots are located on patches of poor fertility. This also leads to an increase in the residual mean square of the analysis of variance, hence to lower precision of the multiple-tree plot design.

- Each tree in a family is growing beside trees of other families, so competition is highly variable and random, as is the pollen environment. This situation comes close to the commercial situation in which trees of different hybrid families are placed in farmers' production plots.
- Knowledge of individual data means that combined "individual-family" selection can be considered; hence it is possible to select a certain number of good trees that might possibly be used in a new selection cycle.
- When the hybrids present have been obtained by a particular mating design, of the NC-II or diallel type, the calculated genetic parameters, such as narrow sense heritability, are parameters estimated on an individual scale.

Disadvantages are:

- As the trees of the different crosses are placed totally randomly in each of the blocks, setting up these trials is laborious, and establishment errors are possible.
- Visual selection of families is difficult or impossible.
- Data have to be collected tree by tree, meaning a heavy workload, high experimental costs and risks of errors.
- The assessment of some traits, such as resistance to rot caused by *Phytophthora* sp., requires a minimum pod yield, which is often not reached for every tree even when results are pooled over several years.

Main statistical methods for improving the selection of individuals

At the outset: mass selection

The first method used for the selection of individual plants in the wild or in production plots was mass selection. This method has been used since the beginning of agriculture. Seeds from the highest yielding plants are collected and sown during subsequent cropping periods. Mass selection is commonly used in cocoa smallholdings; farmers recover seeds from chosen trees to replace missing trees or to set up new plantings. It is also used in some estates to propagate the highest yielding trees, usually by budding. This method has numerous drawbacks: the good performance of a tree may come from a particularly suitable micro-environment, such visual selection usually only takes into account performance over a period of one year, and the pedigree of the candidates for such selection is usually unknown. More efficient selection methods have been proposed with a view to improving the choice of individuals in trial plots.

Three particular methods will be examined: "combined individual-family selection", "spatial statistics" and "data smoothing", notably by the "Papadakis analysis of covariance" or by "longitudinal data analyses". For certain traits and under certain conditions, these last methods make it possible to consider values measured successively over time as replicates.

Combined individual-family selection

This method has been used on several occasions for cocoa to select individuals in mating designs (Cilas *et al.* 1995 and 1999; Ndoumbé *et al.* 2001). Its principle is relatively simple: the value of an individual is not only predicted by its specific performance but also by the performance of the family to which it belongs. In other words, the full-sibs of an individual constitute partial replicates of that individual, as

do its half sibs to a lesser degree. This is the principle of geno-phenotypic regression (Gallais 1989), which is expressed as:

$$\hat{G}_i = G. + \beta(P_i - P.)$$

where:

$\hat{G}_i$ = estimation of the genetic value *G* of tree i,

$G.$ = genetic value of related individuals, and

$\beta = \frac{\text{cov}(G_i, P_i)}{\text{var}\, P_i}$,

P_i = the phenotypic value of tree i, and

$P.$ = the phenotypic value of related individuals.

Apart from enabling a good estimation of genetic parameters for the population from which the parents came, implementing mating designs allows for a more accurate estimation of genetic values for individuals by applying the above formula.

Spatial statistics

One of the most conventional tools in spatial statistics is spatial "autocorrelation", which globally accounts for the tendency of close places to resemble each other (positive autocorrelation) or, conversely, to differ (negative autocorrelation). "Variograms", comparing the scatter of a variable and the distance from a given point, reveal discontinuity in the spatial distribution of a studied phenomenon. Other methods make it possible to take autocorrelation into account in conventional statistical analyses and thereby prevent it from introducing bias in parameter estimations. Of these methods, spatial modeling and data smoothing are the ones most frequently used.

Numerous studies have been conducted to take into account spatial heterogeneity in selection trials (Azais *et al.*. 1990; Bartlett 1978; Baudouin *et al.*. 1987; Besag and Kempton 1986; Gleason and Cullis 1987; Goumari 1990; Kempton and Howes 1981; Pearce 1976; Sébastien 1993). Of these methods, the so-called "Papadakis" method (Papadakis 1937, 1940 and 1984), corresponding to empirical data smoothing, has been more widely used (Dagnélie 1987 and 1989). It is a way of adjusting for environmental effects, applied to individual values, and makes it possible to eliminate an environmental effect for a given trait from the phenotypic value of each individual (individual-pivot). This adjustment is done by multiple regression on a covariable comprising the mean of "residues" of neighbouring individuals for the same trait. The mean relative position of those neighbours in relation to the pivot can be defined by "grids" or neighbourhood configurations. The residuals are defined as the deviations of each individual from the mean of the genotypic class to which it belongs (population, family, clone, *etc.*). Such correction of phenotypic values by consideration of values for a tree's neighbours can be very efficient, for example in analysing field attack by mirids (Brun *et al.* 1997). It has been less efficient for yield itself, possibly because of competition effects between neighbouring trees. In that case, other variables of neighbours may be considered for correction, such as early vigour (indicating soil fertility) or adult vigour (involved in competition between trees).

Other methods have also been developed to estimate competition phenomena (Montagnon *et al.* 2001; Lachenaud and Montagnon 2002).

Longitudinal data analyses: an alternative worth exploring

These are methods used when traits are observed several times on the same individual (Laird and Ware 1982; Verbeke and Molenberghs 2000). The analyses combine multidimensional and temporal aspects. They differ from conventional

multidimensional analyses as the interdependence of observations follows a more structured scheme; they differ from conventional temporal series (a long series of observations on a single individual) through the existence of a large number of short temporal series (one per individual).

Research on methods for processing repeated measurements (or longitudinal data) over time is booming in the field of animal genetics (Foulley *et al.* 2000 ; Rekaya *et al.* 2003), for example the search for a dairy cow genetic evaluation. Data repeated over time, which are generally available when analysing growth, milk production or, in our case, fruit production for a perennial tree crop, are the expression of a potentially infinite number of characters whose co-variances change continuously over time. The characteristic of repeated measurements is that they are often assumed to be correlated with each other as they reflect the expression of the same performance, but at several different moments. Consequently, to carry out an efficient analysis of the data, it is essential to bear in mind how the correlations or co-variances are structured with respect to each other. Longitudinal data analysis therefore concerns all animal species and perennial plant species (Dias *et al.* 1998). Moreover, outside the genetics field, longitudinal data analysis is also a topical research subject in biostatistics.

More powerful experimental designs for choosing individuals

Using twins

A genotype splitting technique at the seed stage was proposed with a view to improving certain cocoa experiments (Bertrand and Cilas 1990). "True twins" were obtained by cleaving cocoa beans longitudinally with a scalpel before sowing. The purpose was to separate the two cotyledons and divide the embryo into two sections. The two half-beans obtained in that way were sown in the usual way. Several experiments were conducted to improve the success rate, with best results obtained by removing the testa before cutting. Using that method, around 30% of the cut beans gave pairs of twins with identical normal development (Bertrand and Cilas 1990). This technique remains to be improved; for example, by pre-germinating the beans beforehand it may be possible to cut the embryo more accurately into two equal parts. The humidity conditions and the substrate for germination could also be studied, with a view to minimising rotting and drying out of the half seeds.

Using true twins in genetic trials would make selection for the genotypic value in breeding plots more efficient. Indeed, each genotype would be represented twice, making it possible to break down residual variance into environmental variance and full-sib within-family variance. Following the analysis of the statistical differences between the means of crosses and parents, it would then be possible also to statistically classify genotypes within families. An additional advantage of this technique would be that all the trees in comparisons would come from seed and would therefore have the same architecture, with an orthotropic stem and a canopy of plagiotropic branches.

Other early propagation techniques of seedlings

If the main purpose of a trial is to select new individuals from within progenies, vegetative propagation of the individuals can be considered at an early stage, so as to plant the different replicates of the genotypes in trials with an appropriate statistical design. This method is more laborious, but may be worth the effort to improve and speed up selection progress for special purposes. A technique can be applied to propagate seedlings in the nursery (by orthotropic cuttings, buddings or grafts onto seedling rootstocks) or one can decide to wait for the individuals to develop a jorquette before vegetative propagation and then use plagiotropic branches.

Seedlings may also be manipulated (*e.g.* bending) to obtain a larger number of orthotropic stems for multiplication (Améfia *et al.* 1985). Somatic embryogenesis could be another alternative, when facilities are available, but since the currently available techniques require floral parts, it would not represent an early multiplication method.

Budding or grafting remains probably the most appropriate technique for rapid multiplication of nursery seedlings. This technique has been used at CCRI in Papua New Guinea precisely for setting up a progeny trial with replicated individuals (see Efron *et al.*, proceedings of this workshop). However, using this technique to select genotypes presupposes that there is no rootstock x scion interaction that will disrupt the genetic value of the scions and thereby the classification of genotypes based on the traits observed. Moreover, these different propagation methods may induce phenotypic variability through irregular outgrowth of the budded or grafted scions or of the rooted cuttings.

Conclusion

Several statistical methods are available for improving the selection of new genotypes (individual trees, with or without multiplication) in breeding trials comprising full-sib populations. Combined individual-family selection provides a more accurate prediction of an individual by taking into account the genetic relationship between trees in the trial. This method can be improved by taking into account any spatial effects that might exist in trial plots. If spatial effects are detected, data smoothing can prove effective. Data smoothing can be achieved with a spatial model or by an empirical method such as the Papadakis method. When trials are monitored over several years, longitudinal data analysis might also enable a more accurate estimation of individual genetic values.

If the purpose of a trial is in fact to select individuals, appropriate experimental designs can be set up. Different vegetative propagation methods can be used to have replicates of each genotype. However, most of these methods are quite laborious and care should be taken not to induce other sources of heterogeneity. Using true twins obtained by cleaving cocoa seeds, once fully mastered, is recommended for rapid duplication of genotypes to be planted in trials. A partial diallel-type mating design, in which individuals are duplicated by this seed cleavage system, would be very powerful for estimating genetic parameters and choosing genotypes in field trials.

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Assessment of the Yield of Individual Cacao Trees in Four Field Trials

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Abstract

In four field trials of Upper Amazon cacao hybrids 16.8 - 27.2% of the cacao trees produced no useable pods, 38.8-66.3% yielded 1-10 pods and 6.8-38.0% had more than 10 pods per year. Some trees of T60/887 progenies were very high yielding and produced up to 180 pods per year. Tree-to-tree variation in yield was very high (C.V. = 30.2 - 76.0%). Heritabilities for individual tree yield were very low, hence it is recognised that this variation is partly due to environmental and partly due to genetic factors. In our study, individual tree yield was highly correlated with stem girth, suggesting that inter-tree competition may be involved. There were no significant differences between the yields of the control crosses at the four trial sites, indicating that there was very little genotype x environment interaction. The results indicate that breeders should aim at a high proportion of individual trees contributing to yield in any selected variety. This could be improved, on one side, by using more uniform cacao varieties (*i.e.* clones or genetically uniform hybrid varieties). On the other side, environmental effects could be reduced by applying good and uniform agronomic practices, which might include the thinning of densely planted cacao stands at adult age to reduce inter-tree competition.

Introduction

One of the generally observed characteristics of cacao is the high degree of yield variation among trees (Figueira and Janick 1995). A recent study of the early yield of five high-producing cacao families grown in full sun in Puerto Rico showed that 2 to 3% of the trees in a population accounted for more than 60% of the yield (Irizarry and Rivera 1998), indicating that most of the trees were low-yielding. Such wide tree-to-tree variations in performance could be a major factor in poor productivity especially when the largest proportion of trees is contributing very little to the overall yield. Wide tree-to-tree variation is commonly observed in cacao stands derived from seeds. Though plant breeding and selection can attain improvement in yield as a result of additive genetic gain, the gain is lost especially as farmers use F2 seed for their own new plantings, thus increasing genetic variation and tree-to-tree variation as well.

Vegetative propagation systems can provide a means to capture additive and dominant genetic variation (Maximova *et al.* 2002). However, in West Africa, including Ghana, cacao has traditionally been planted from seed on smallholdings, mainly because it is easier and cheaper to establish these from seeds than from vegetatively propagated plants. On these smallholdings, yields are around 300 kg/ha of dry cocoa beans (Smith 1994). This contrasts with the net yields ranging from 124 to 966 kg ha^{-1} (an average of around 600 kg ha^{-1}) that are observed in Ghana in breeding trials of Upper Amazon cacao hybrids carried out under conditions that resemble farmers' fields (Adomako and Adu-Ampomah 2000).

Kearsey and Pooni (1996) cautioned that for many developing countries there could be major differences between the breeders' environment and those that prevail in farmers' fields. Thus if varieties that are selected in breeders' trials are not stable, they may perform relatively badly on farms under different environmental conditions. Ideally, if a breeder wishes to produce a variety that performs well in a particular environment, then selection should be carried out in that environment. In practice, however, this is not what prevails.

The present paper analyses the yields of individual trees and their contribution to yield in four field trials of Upper Amazon cacao hybrids established in CRIG field plots at Tafo and Apedwa in the eastern region of Ghana (all carried out under conditions that resemble farmers' fields). The possible implications of the results for upgrading yields in farms with mature bearing cacao trees are discussed.

Material and methods

The four trials, namely the 25 th, 26 th, 27 th and 28 th progeny trials, were established in May/June 1983, 1985, 1989, and 1991, respectively. The 25 th and 26 th progeny trials were planted at Tafo, while the 27 th and 28 th progeny trials were located at Apedwa (15 km from Tafo). The experimental design for all the trials was randomised complete block with five replications and 25 trees per plot. The cacao trees were spaced at 2.5m x 2.5m and grown under shade of mixed species of forest trees, supplemented with *Gliricidia sepium* where necessary (this was to simulate conditions in a typical West African cacao farm). Insecticides were used to control insect damage but neither fungicides nor fertilisers were applied. Two hybrid varieties, T85/799 x Amel and T85/799 x T79/501, were included in each trial as standards for comparison because of their high yield and low losses from black pod disease in earlier series of trials (Lockwood 1976). All the cacao trees were planted from seedlings obtained from hand pollinated pods. The origin and parentage of the cacao selections used in the trials are given by Lockwood and Gyamfi (1979).

Data recorded included number of pods harvested at approximately three-week intervals classified into six categories: healthy ripe, black pod infected and ripe, black pod infected and unripe, rodent damaged, empty and otherwise damaged. The yield data (kg/ha dry cacao beans) presented are based on the mean values of ripe pods containing useable beans (fresh beans that are suitable for fermentation) recorded for five cropping years (1995/96 - 1999/2000). Rodent damage was generally less than 2%, black pod incidence was below 7% and discards at breaking were less than 10% (data on these are not presented). Trunk girth was measured 15 cm above leaf litter eight years after planting.

Correlation studies used coefficient of linear correlation (Pearson) and heritability values were estimated as shown by Simmonds (1979).

Results and discussion

Pods per tree

The yields of individual trees in the 25 th, 26 th, 27 th and 28 th progeny trial areas (PTAs) are presented in Tables 1, 2, 3 and 4 respectively. In the 25 th PTA, the highest yields of 16.0, 15.8, 15.8, and 15.5 pods per tree per year were from crosses of T60/887 with POUND 10, Be 8, POUND 15 and Rb 49, respectively. The yields were, however, not

significantly different from those produced by the control crosses. The highest yielding crosses from the 26 th, 27 th and 28 th PTAs were T85/799 x PA 150 (14.9 pods/tree), T85/799 x CAS 3 (10.0 pods/tree), and Pa 150 x Amaz 3-2 (9.8 pods/tree). PA 150 appears to be a good parent for yield when used either as the female or male parent in crosses.

The yields of crosses in the 25 th PTA, including the controls, were generally higher than those recorded for the crosses in the other trials. This may be because the 25 th PTA is the oldest of the four trials and also because T60/887 has a good general combining ability for yield (Adomako *et al.* 1999).

The ratios of the yields of T85/799 x Amelonado (Control 1) and T85/799 x T79/501 (Control 2) in the four trials are 1.1 : 1.0 (25 th PTA), 0.9 : 1.0 (26 th PTA), 0.9 : 1.0 (27 th PTA) and 1.2 : 1.0 (28 th PTA) which is approximately 1 : 1 in each of the four trials. There was also no significant difference in yield between the two controls in each of the four trials, although control 1 was higher yielding than control 2 in all the trial sites. This may suggest that there was very little genotype by environment (G x E) interaction occurring in these trials.

Yield distribution, range and coefficient of variation

In the 25 th PTA a mean of 16.8% of the trees produced no useable pods per year. T60/887 x Rb 49 had the lowest percentage (7.1%) whilst T60/887 x EQX 3338 gave the highest value of 23.7% which was significantly higher than the percentages for the two controls (Table 1). Generally, crosses of T60/887 with the EQX pollen (male) parents gave significantly higher percentages of trees with no useable pods than the controls except the cross with EQX 3356 which had a value similar to the control T85/799 x T79/501. Higher percentages of trees with no useable pods were recorded in the 26th, 27th and 28th PTAs than in the 25 th PTA. The 26 th, 27 th and 28 th PTAs had 25.5%, 27.2% and 26.3%, respectively (Tables 2, 3 and 4) and more than 50% of the trees in these trials produced only 1-10 pods per year. Again, in the 25 th PTA (the highest yielding trial) 45.0% of the trees yielded more than 10 pods per year (Table 1) whilst in the 27 th PTA (the lowest yielding trial) only 6.8% produced more than 10 pods per year (Table 3).

The largest range (0-180) of pods produced per year was observed in the 25 th PTA and the lowest (0-26) was recorded in the 27 th PTA. Tree-to-tree variation in yield as measured by the coefficient of variation (C.V) was very high (30.2 - 76.0%) which agrees with observations made by Figueiro and Janic (1995). The lowest C.V. of 30.2% was observed in the cross T85/799 x Pa7 in the 26 th PTA (Table 2) and the highest value of 76.0% was given by PA 150 x CC 11 in the 28 th PTA (Table 4).

Heritability and correlations

Heritability (h^2) values for yield were 0.084, 0.058, 0.045, and 0.046 for progenies in the 25th, 26th, 27th and 28th PTAs, respectively. The h^2 values were very low which indicates that genetic effects were less important than environmental ones on yield. Also, the coefficients of correlation (r) between individual tree yield and girth were highly significant in all the trials (r = 0.75, 0.67, 0.58, and 0.76 for the 25th, 26 th 27 th and 28 th PTAs, respectively). This suggests that much of the variation in yield was due to variation in vigour, which agrees with the observation by Sounigo *et al.* (2004) that inter-tree variation in cacao is mainly due to environmental factors. Lachenaud (2004) also observed that inter-tree variation in cacao is mainly due to competition between trees and suggested that thinning of one tree out of two will decrease competition and enhance individual tree yields.

General discussion

Although adequate information on the performance of individual cacao trees in farmers' fields in Ghana is not available, it is reasonable to conclude that the situation on these farms could be the same or even worse in old farms, than in the breeding trials. A greater percentage of cacao trees in farms may not be producing any useable pods during the cropping season.

The yields of cacao in farmers' fields can be increased significantly if unproductive trees are replaced with high yielding ones. Purdy and Eskes (2002) suggested that top-working of old trees, grafting or budding chupons of old trees, and any other methods that use the roots of old cacao trees are quick ways of rectifying poor performance provided environmental conditions are favourable. Side-grafting is used most commonly in Malaysia to upgrade the production of poor yielding or non-bearing mature trees that are already planted in the fields (Yow and Lim 1994). Farmers in West Africa can also employ the side-grafting technique to upgrade yields on their farms after identifying the unproductive trees in their fields. Glicenstein *et al.* (1990) recommended that it would be much more desirable to obtain budwood for grafting from orthotropic (chupon) rather than from plagiotropic (fan branch) material because trees originating from orthotropic material grow in the upright, more easily manageable pattern characteristic of seed-derived trees.

The yields from the 25th PTA, including the controls, were generally higher than those recorded from the other trials. This is an indication of environmental effect on yield. However, there appeared to be no significant genotype x environment interaction effect on yield since there was no significant difference in yield between the two control crosses in all the four trials, although control 1 was higher yielding than control 2 in all the trials. Again the ratio of the yields of the two controls was approximately 1:1 in each of the four trials. Thus, it could be said that the control crosses were stable in yield. The results imply that the best materials for high yield selected in one environment would give relatively high yields in any other environment provided that the environment is generally suitable for cacao cultivation.

The poor yields of most of the cacao varieties in the field might be partly attributed to the high percentage of trees that produce no useable pods during the cropping season. Any quantitative trait identified at the seedling stage, which is linked to high yielding ability, would greatly aid breeding and selection of high yielding cacao varieties. In the absence of such a quantitative trait, and because breeding for yield would be much more difficult than improving growing conditions, side-grafting and thinning can be used to improve the yields of non-bearing or poor yielding trees on farms. In addition, farmers in West Africa should be encouraged to use vegetative propagation in new plantings instead of F2 seeds so that the genetic gain in yield achieved by breeding and selection could be attained on their farms.

Conclusions

As a result of the low heritability for yield, it appears that breeding for yield would be difficult. The best way would probably be to use clones to obtain maximum genetic gains. Improving environmental conditions would be a rapid and effective way to improve yields of existing poor-yielding or non-bearing mature trees in farmers' fields in the short-term.

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Table 1. Mean, distribution, range and coefficient of variation (C.V) of individual tree yields in the 25th PTA based on average yields per year

Progenies	Mean yield		Percentage of plants producing			Range	Tree-to-tree C.V (%) for yield	Mean trunk girth (cm)
	Useable pods	Kg/ha dry wt. of beans	0 pods	1-10 pods	> 10 pods			
T60/887 x POUND 10	16.0f	1075.4	9.3ab	33.9b	56.8k	0-68	34.5a	43.4
T60/887 x BE 8	15.8f	1061.9	17.9defgh	31.3a	50.8ghi	0-88	35.3ab	44.0
T60/887 x POUND 15	15.8f	1061.9	10.7abc	38.4abc	50.9ghi	0-81	52.1def	42.4
T60/887 x RB 49	15.5f	1041.8	7.2a	41.1bcd	51.7hij	0-130	39.4abcde	38.5
T60/887 x Amelonado	14.9ef	1001.5	15.4bcdef	38.5abc	46.1efg	0-180	38.4abc	41.2
T60/887 x POUND 21	14.6def	981.3	22.4gh	31.2a	46.4fg	0-118	52.5defg	40.8
T60/887 x AMAZ 3-2	14.5cdef	974.6	16.3cdefg	32.0a	51.7hij	0-67	36.6ab	40.8
T60/887 x ICS 6	14.2bcdef	954.4	18.9defgh	34.1ab	47.0fgh	0-146	41.9abcdef	40.6
T60/887 x Ma 12	13.9bcdef	934.2	14.7bcde	41.1bcd	44.2def	0-72	37.8abc	40.5
T60/887 x T17/524	12.0abcde	806.5	17.1cdef	42.1cd	40.8cd	0-74	41.3abcdef	36.9
T60/887 x EQX 3356	11.7abcd	786.4	19.7defgh	46.7d	33.6a	0-83	50.2cdef	38.8
T60/887 x CATONGO	11.7abcd	786.4	22.1fgh	37.6abc	40.3bcd	0-94	54.2efg	41.3
T60/887 x IMC 60	11.7abcd	786.4	17.1cdefg	41.6b	41.3cde	0-64	43.1abcde	38.1
T60/887 x EQX 3338	11.5abcd	772.9	23.7h	36.8abc	39.5bcd	0-83	54.3efg	40.2
T60/887 x EQX 3364	11.4ab	766.2	20.3efgh	41.5bcd	38.2abc	0-72	48.7bcdef	38.1
T60/887 x C-SUL 7	11.3ab	759.5	20.8efgh	43.5cd	35.7ab	0-105	65.6g	36.5
T60/887 x POUND 7	10.7ab	719.2	16.5cdefg	46.7d	36.8abc	0-67	34.7a	34.2
T60/887 x IMC 78	10.0a	672.1	20.0efgh	42.0cd	38.0abc	0-73	55.5fg	35.0
T85/799 x Amelonado (C1)	16.3f	1095.5	13.0abc	31.1a	55.9jk	0-90	39.6abcd	40.1
T85/799 x T79/501 (C2)	14.6def	981.3	13.2abcd	32.9ab	53.9ij	0-88	36.1ab	41.0
Mean	13.4	900.8	16.8	38.2	45.0		44.6	
Standard error	1.45	97.41	3.16	3.60	5.04		6.27	
Range	10.6-16.0	673.1-1095.5	7.2-23.7	31.1-46.7	33.6-56.8	0-180	34.3-65.6	34.2-44.0
C.V.(%) between progenies	15.3	15.3	26.6	13.3	15.9			

C1, C2: control (standard) crosses included for comparison.
For each column, figures followed by the same letter are not significantly different at the 0.05 level.

Table 2. Mean, distribution, range and coefficient of variation (C.V) of annual individual tree yields in the 26th PTA based on average yields per year

Progenies	Mean yield		Percentage of plants producing			Range	Tree-to-tree C.V (%) for yield	Mean trunk girth (cm)
	Useable pods	Kg/ha dry wt. of beans	0 pods	1-10 pods	> 10 pods			
T85/799 x PA 150	14.9e	1001.5	21.1abc	45.8ab	33.1de	0-60	49.1cd	41.3
T85/799 x Ma 12	14.7de	988.0	19.2ab	48.4abc	32.4de	0-62	48.0cd	42.1
T85/799 x PA7	14.1de	947.7	18.5a	44.8ab	36.7e	0-43	30.2a	38.7
T85/799 x PA 107	13.6cde	914.1	26.0bcde	41.0a	33.0de	0-60	63.6e	36.9
T85/799 x SCA 9	12.5bcde	840.1	19.9abc	56.2cde	23.9bcd	0-87	50.2cde	37.9
T85/799 x Alph.B36	11.7bcd	786.4	28.2de	50.8bcd	21.0abcd	0-76	52.2cde	38.4
T85/799 x IMC 78	10.7abc	719.2	28.1de	52.3bcde	19.6abc	0-64	43.1abc	39.6
T85/799 x NA 440	10.6abc	712.4	29.5de	52.3bcde	18.2abc	0-67	61.1de	34.0
T85/799 x T44/547	10.0ab	672.1	32.1e	49.4bc	18.5abc	0-54	43.5abc	32.1
T85/799 x GA 11	9.9ab	665.4	27.5cde	59.6e	12.9ab	0-39	45.0bc	38.4
T85/799 x IMC 23	8.6a	578.0	30.5de	58.1de	11.4a	0-27	49.3cd	32.1
T85/799 x Amelonado (C1)	12.1bcde	813.3	24.3abd	50.0bc	25.7cde	0-60	51.2cde	33.7
T85/799 x T79/501 (C2)	10.9abc	732.6	29.1de	48.8abc	22.1abcd	0-43	35.4ab	38.4
Mean	11.9	797.8	25.5	50.6	23.9		47.8	
Standard error	1.41	94.7	3.20	3.73	5.68		6.39	
Range	9.9-14.9	578.0-1001.5	18.5-32.1	41.0-59.6	11.4-36.7	0-87	30.2-63.6	32.1-42.1
C.V (%) between progenies	16.8	16.8	18.0	10.4	33.9			

C1, C2: control (standard) crosses included for comparison.
For each column, figures followed by the same letter are not significantly different at the 0.05 level.

Table 3. Mean, distribution, range and coefficient of variation (C.V) of annual individual tree yields in the 27 th PTA based on average yields per year

Progenies	Mean yield		Percentage of plants producing			Range	Tree-to-tree C.V (%) for yield	Mean trunk girth (cm)
	Useable pods	Kg/ha dry wt. of beans	0 pods	1-10 pods	> 10 pods			
T85/799 x CAS 3	10.0h	672.1	22.4abcdef	57.6ab	20.8e	0-21	57.5bcd	43.0
T85/799 x UF 713	9.6gh	645.2	32.0defg	56.0ab	12.0d	0-23	69.4ef	42.4
T85/799 x P4A	9.0fgh	604.9	18.2abcd	71.2bcd	10.6cd	0-21	49.0ab	42.2
T85/799 x Catongo	8.9efgh	598.2	22.4abcdef	69.6bcd	8.0abcd	0-11	49.0ab	39.0
T85/799 x PA7	8.8defgh	591.5	12.8ab	77.6cd	9.6bcd	0-21	52.8abc	42.4
T85/799 x Be8	8.7defgh	584.7	16.0abc	83.2cd	0.8a	0-13	56.4bcd	38.6
T85/799 x MOQ 647	8.7defgh	584.7	12.8ab	83.2cd	4.0abc	0-17	49.9ab	40.5
T85/799 x SIAL 93	8.6cdefgh	578.0	35.2fgh	52.8a	12.0d	0-15	63.7cdef	38.6
T85/799 x EEG 8	8.5cdefgh	571.3	28.8cdefgh	67.2abc	4.0abc	0-23	61.4bcdef	40.1
T85/799 x CC 10	8.3 cdefgh	557.9	25.6bcdefg	66.4abc	8.0abcd	0-16	62.3bcdef	37.1
T85/799 x AMAZ 3-2	7.6 abcdef	510.8	40.0gh	59.5a	0.5a	0-11	40.1a	33.3
T85/799 x SC 5	7.5abcde	504.1	32.0defgh	66.0abc	2.0ab	0-13	58.2bcd	40.0
T85/799 x EET 377	7.4abcde	497.4	34.2efgh	60.5ab	5.3abcd	0-18	74.9ef	36.6
T85/799 x 10P	7.2abc	483.9	38.4gh	57.6ab	4.0abc	0-11	75.5f	35.0
T85/799 x UF 211	7.2abc	483.9	41.6h	58.0ab	0.4a	0-9	60.5bcd	39.9
T85/799 x SPA 7	7.0ab	470.5	40.0gh	58.0ab	2.0ab	0-9	48.3ab	35.6
T85/799 x SPA 10	6.5a	436.9	35.2fgh	52.8a	12.0d	0-9	69.8def	41.2
T85/799 x Amelonado (C1)	8.9efgh	537.7	9.6a	84.4d	8.8bcd	0-26	57.3bcd	43.8
T85/799 x T79/501 (C2)	8.0bcdefg	598.8	19.2abcde	72.0bcd	6.0babcd	0-21	60.6bcd	44.0
Mean	8.2	553.2	27.2	66.0	6.8		58.8	
Standard error	0.66	45.50	7.32	7.36	3.63		6.66	
Range	6.5-10.0	436.9-672.1	9.6-41.6	52.8-84.4	0.4-20.0	0-26	40.1-75.5	33.3-44.0
C.V (%) between progenies	11.4	11.4	38.1	15.7	75.0			

C1, C2: Control (Standard) crosses included for comparison
For each column, figures followed by the same letter are not significantly different at the 0.05 level.

Table 4. Mean, distribution, range and coefficient of variation (C.V) of annual individual tree yields in the 28th PTA based on average yields per year

Progenies	Mean yield: Useable pods	Mean yield: Kg/ha dry wt. of beans	Percentage of plants producing: 0 pods	Percentage of plants producing: 1-10 pods	Percentage of plants producing: > 10 pods	Range	Tree-to-tree C.V (%) for yield	Mean trunk girth (cm)
PA 150 x AMAZ 3-2	9.8ef	658.7	28.5cdef	60.0abc	11.5f	0-23	58.0ab	42.1
PA 150 x Catongo	9.5de	638.5	24.0abc	66.5bcde	9.5cdef	0-26	52.9ab	42.6
PA 150 x SGU 89	9.5de	638.5	24.0abc	64.0abcd	12.0f	0-22	50.7ab	42.2
PA 150 x P11B	9.2cde	618.3	19.0a	78.0f	3.0ab	0-24	58.0ab	40.0
PA 150 x CC 11	9.2cde	618.3	32.0ef	56.0a	12.0f	0-25	76.0c	41.1
PA 150 x IMC 78	9.2cde	618.3	31.0def	58.6abc	10.4ef	0-24	52.9ab	38.9
PA 150 x UF 667	8.9bcde	598.2	23.9abc	71.1def	5.0abcd	0-34	56.6ab	38.5
PA 150 x EET 399	8.8bcde	591.5	32.0ef	58.0ab	10.0def	0-26	57.9ab	37.8
PA 150 x RB 49	8.6abcd	578.0	26.7bcde	65.4bcde	7.9cdef	0-25	53.6ab	35.0
PA 150 x CC 38	8.6abcd	578.0	23.9abc	71.9def	4.2ab	0-33	51.5ab	38.6
PA 150 x BE 8	8.5abcd	571.5	21.6ab	73.7ef	4.7abcd	0-12	46.5a	34.6
PA 150 x EQX 3364	8.3abc	557.9	27.0bcdef	70.0cdef	3.0ab	0-16	53.0ab	40.5
PA 150 x IMC 60	8.3abc	557.9	33.5f	61.5abc	5.0abcd	0-31	62.8bc	34.5
PA 150 x C-SUL 7	8.1ab	544.4	31.0def	63.5abcd	5.5abcde	0-17	58.4ab	32.8
PA 150 x IMC 23	7.7a	517.5	27.5bcdef	71.0def	1.5a	0-16	52.9ab	38.1
T85/799 x Amelonado (C1)	9.7def	656.2	21.0ab	67.5cde	11.5f	0-27	51.4ab	40.0
T85/799 x T79/501 (C2)	8.7abcd	584.7	25.0abcd	67.6cde	7.4bcdef	0-27	49.8ab	40.5
Mean	8.9	599.4	26.3	66.3	7.4		55.5	
Standard error	0.51	36.07	3.08	4.23	2.46		6.41	
Range	7.7-10.7	517.5-719.2	19.0-33.5	56.0-78.0	15-12.0	0-34	46.5-76.0	32.8-42.6
C.V. (%) between progenies	8.0	8.0	16.6	9.0	47.0			

C1, C2: Control (Standard) crosses included for comparison
For each column figures followed by the same letter not significantly different at the 0.05 level

Analysis of the Factors Affecting Yield and Yield Variability in the SG2 Cocoa Hybrid Variety in Papua New Guinea

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Abstract

The SG2 hybrid is the most widely grown cocoa variety in Papua New Guinea (PNG). It was originally released as a poly-cross hybrid of 15 different crosses. In 1994, it was modified to include only 10 crosses in two groups (SG2-B and SG2-S) of 5 crosses each based on their potential vigour (bigger and smaller trees). It is characterised by a high degree of tree-to-tree variability in yield. A relatively high proportion of the trees is low yielding, and called 'passenger trees' by the farmers.

An experiment aimed at increasing our understanding of the reasons for the production variability between and within crosses was initiated in 1996. Forty trees of each of the 10 SG2 hybrid crosses were planted. Variables associated with pod production were measured from January 1999. A wide range of variability in the number of flowers and pollination efficiency was found between and within crosses. The pollination efficiency was negatively correlated with the number of flowers. The number of cherelles produced also varied between and within crosses, such that about 70% wilted before growing to 3-5 cm long. There were no apparent genetic differences between the 10 SG2 crosses for the proportion of cherelle wilt. However, the proportion of cherelle wilt was closely associated with the rainfall. As the rainfall increased, the proportion of wilted cherelles decreased.

The tree-to-tree variability in the number of pods per tree was confirmed. A comparison between low and high yielding trees has shown that higher yielding trees were more efficient in converting flowers into pods mainly due to events that occur at the late cherelle stage. A better understanding of the mechanisms involved would assist the breeder in developing higher yielding cocoa genotypes.

Introduction

Two polypro Trinitario x Upper Amazonian cocoa hybrid varieties, SG1 and SG2, were released in Papua New Guinea (PNG) in 1982 and 1988, respectively. The hybrids were released as being higher yielding and more precocious than the previously grown Trinitario variety, which was mostly derived from open pollinated pods. The release was followed by wide scale adoption of the hybrids by large and small-scale growers. However, during the early 1990s, it was noted that the high expectations of the hybrids had not been fully realised. Two major problems were encountered:

1) tree-to-tree variability in pod production, including the existence of a high proportion of less productive trees, named 'passenger trees' by the local farmers; and
2) yield decline that starts about 4-5 years after initial production.

In 1996, the Cocoa and Coconut Research Institute (CCRI) of PNG initiated research, part of which is reported in this paper, in an attempt to understand the factors responsible for the yield variability and yield decline in the SG2 hybrid.

Cocoa yield at a given density depends on the number of pods produced per tree, pod weight, the percentage of wet beans in the pod and the conversion rate of

wet to dry beans after fermentation. The number of pods per tree is a product of the number of flowers produced, flower setting (pollination) and the events occurring during cherelle and fruit development. Each of these stages is a complex phenomenon, resulting from interaction between the genotype of the tree and its environment. Flowering, pollination and fruit development, particularly cherelle wilt, were subjects of many investigations, which have been reviewed by Alvim (1984) and Young (1986). The focus in the present study was the genetic variability between and within crosses of the SG2 hybrid grown in PNG.

Materials and methods

Commercially produced seeds of the ten SG2 crosses (Table 1) were used. KEE designates locally selected Upper Amazonian clones used as females, whilst K82 and KA2-106 are local Trinitario clones used as males. The cross KEE 43 x KA2-106 (?) was discovered to be of the wrong identity.

Forty trees of each cross were field planted in October 1996 in one replication (five rows, eight trees per row), to minimise the environmental variability within the cross, at a density of 625 trees ha^{-1}. Flower counting was initiated in January 1999. A 2 m^2 piece of shade cloth was placed, supported about 40 cm above the ground, under each tree. The flowers that fell on the shade cloth were collected and counted twice a week.

Cherelles on the portion of the tree above the shade cloth were counted once a month. The wilted cherelles were removed from the tree and separated into black and yellow cherelles. The healthy green cherelles were counted on the trees. To avoid counting the same cherelles twice, only cherelles that were 3-5 cm long were counted. Mature pods were harvested and counted fortnightly for each tree. Pod and wet bean weights were measured for each cross (not individual trees) by weighing all the mature pods harvested from each cross on any particular date. A common conversion rate of 0.3 was used to convert wet bean weight to dry bean weight.

The data obtained for 1999 and 2000 were summarised quarterly and annually for individual trees and as a cross average. Some of the trees that started to flower after January 1st, 1999 were not included with the 1999 data. The following variables were calculated:

1) Number of flowers and cherelles = number of flowers and cherelles (of 3-5 cm long) counted.
2) Pollination efficiency (%) = number of cherelles/number of flowers x 100.
3) Proportion of green cherelles = number of green cherelles / number of green plus wilted (black) cherelles x 100.
4) Expected number of pods = (number of flowers x pollination efficiency/100) x (proportion of green cherelles/100).
5) Actual: expected pod ratio (%) = number of pods harvested/number of expected pods x 100.
6) Pods: flowers ratio (%) = number of pods harvested at six months (the time from flowering to pod maturity) after flowering/number of flowers x 100.

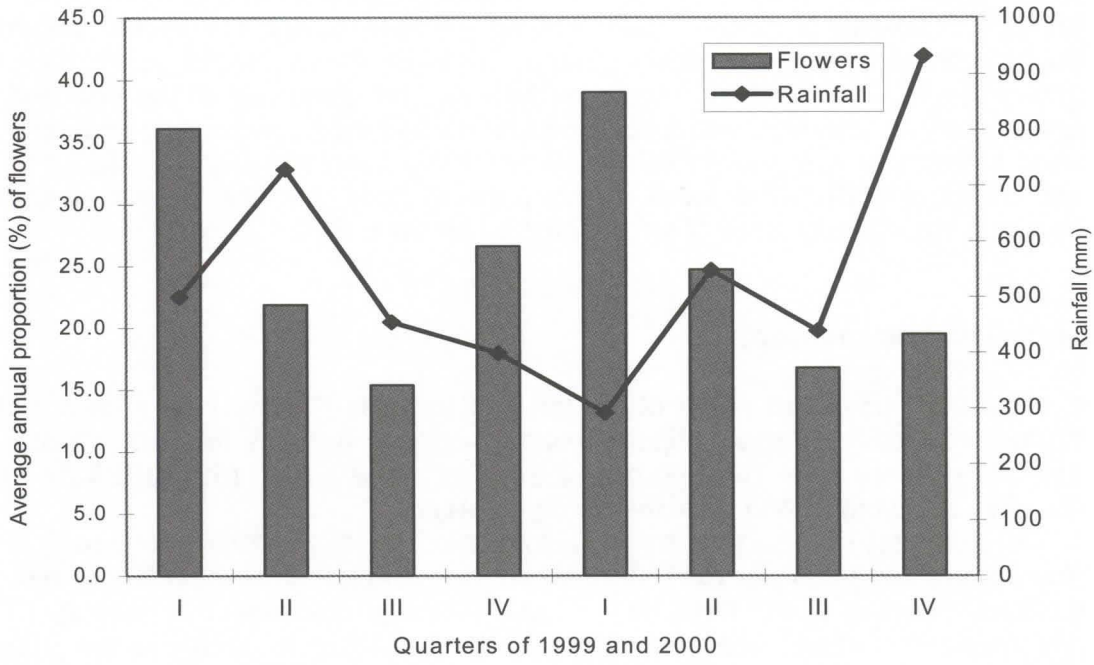

Figure 1. Average quarterly proportion of flowers and rainfall of ten SG2 hybrid crosses during 1999 and 2000

For the comparison between high and low yielding trees, the four trees from each cross with the highest number of pods and the four trees from each cross with lowest number of pods/tree were selected. Very low yielding trees with less than 25 pods/tree were not selected to avoid those suffering possible environmental stresses such as water logging, insect damage, *etc*. The proportional difference between the two groups was calculated as (1 – low/high) x 100.

Results

Flowering and pollination efficiency

Flowering occurred throughout the year in both 1999 and 2000, which is typical of young cocoa trees in PNG. However, flowering intensity was different (Figure 1). In both years, there were sharp peaks of flowering in the first quarter. The number of flowers was lower during the second quarter and decreased further during the third quarter. An increase started in the fourth quarter. All the ten crosses showed a very similar pattern of flowering that did not appear to be associated with the rainfall.

The average annual number of flowers/tree/2m^2 varied greatly between crosses, ranging from 652 in KEE 42 x K82 to 2120 in KEE 47 x KA2-106 (Table 1). More flowers were produced in 1999 than in 2000 (an average of 1469 and 942 flowers/tree, respectively) but the rankings of the ten crosses during the two years were almost identical. The results of the questionable cross, KEE 43 x KA2-106, were similar to those of KEE 5 x K82. A very wide range of variation was also observed for the number of flowers/tree within all the crosses, as indicated by the range between the lowest and highest flowering trees.

Table 1. Average annual number of flowers/tree, pollination efficiencies and number of pods/tree of the ten SG2 TA crosses during 1999 and 2000

TA cross no.	Pedigree	Flowers/ tree		Pollination efficiency		No. pods/ tree[2)]	r_1	r_2
		Number[1)]	Range	(%)[1)]	Range			
38	KEE 47 x KA2-106	2120 (1,1)	676-3632	22.8 (9,10)	8-29	50.2	-0.68**	-0.32 n.s
35	KEE 23 x KA2-106	1664 (2,2)	582-2141	28.9 (8,7)	14-46	49.0	-0.68**	-0.35 n.s
33	KEE 12 x KA2-106	1383 (3,3)	557-2195	26.6 (7,9)	9-43	64.8	-0.46**	-0.34*
31	KEE 5 x KA2-106	1298 (4,4)	576-2060	33.9 (5,6)	17-51	69.4	-0.66**	-0.49**
13	KEE 12 x K82	1089 (5,7)	474-1153	42.0 (1,1)	24-58	74.8	-0.49**	-0.21 n.s
36	KEE 42 x KA2-106	1077 (6,5)	485-1989	25.3 (10,8)	12-50	50.3	-0.66**	-0.23 n.s
17	KEE 43 x K82	1006 (7,6)	324-1778	37.8 (3,4)	20-53	55.1	-0.79**	-0.53**
11	KEE 5 x K82	800 (8,8)	433-1572	36.2 (4,3)	26-51	64.3	-0.58**	-0.43**
37	KEE 43 x KA2-106 (?)	735 (9,9)	544-1062	41.4 (2,2)	28-52	62.9	-0.29 n.s	-0.59**
16	KEE 42 x K82	652 (10,10)	457-1011	33.5 (6,5)	28-49	49.3	-0.38*	-0.44**

1) The number in brackets represents the rank in 1999 and 2000, respectively.
2) Average annual number of pods during the period of July 1999 to June 2001 (six months after flowering).
r_1 Correlation coefficients (within crosses) between the number of flowers and pollination efficiency.
r_2 Correlation coefficients (within crosses) between the number of flowers and the number of pods harvested six months later.
*,** significant at the 5% and 1% levels, respectively.

A parental effect was also found for flowering intensity (Table 2). Among the Trinitario parents, KA2-106 was a donor for higher numbers of flowers than K82. KEE 12 and KEE 42 were donors for the highest and lowest numbers of flowers, respectively among the Upper Amazonian clones. The Upper Amazonian clones KEE 23 and KEE 47 were not crossed with K82. However, they produced the highest number of flowers among the crosses with KA2-106 (Table 1) and therefore, they should be considered as donors for the highest number of flowers among the Upper Amazonian clones, particularly KEE 47.

As noted in the study of number of flowers, a wide range of variation between and within crosses was also observed for pollination efficiency (Table 1). It ranged from an average of 22.8% (KEE 47 x KA2-106) to 42.0% (KEE 12 x K82). The range within the crosses was even greater. Among the parental clones, K82 and KEE 42 were donors for the highest and lowest pollination efficiencies, respectively (Table 2).

The average pollination efficiency of the ten crosses varied throughout 1999 and 2000 (Figure 2). It was higher in the third quarter of 1999 and the third quarter of 2000, the periods with the lowest numbers of flowers (Figure 1). The crosses TA 36 (KEE 42 x KA2-106) and TA 13 (KEE 12 x K82) followed a similar pattern to the average of the ten crosses, but at a different magnitude, such that TA 13 (KEE 5 x K82) had consistently higher pollination efficiencies than TA 36. The pollination efficiency of TA 38 in 1999 did not follow the same pattern. It was more uniform throughout the year without the sharp peak in the third quarter.

Table 2. Average effect of two Trinitario and three Upper Amazonian parental clones on the number of flowers, pollination efficiency and number of pods

Clone	No. of flowers/tree	Pollination efficiency (%)	No. of pods/tree
KA2-106 (T)	1253	28.6	61.5
K82 (T)	847	37.2	62.8
KEE 5 (UA)	1049	35.0	66.9
KEE 12 (UA)	1236	34.3	69.8
KEE 42 (UA)	864	29.4	49.8

T = Trinitario UA = Upper Amazonian

Flowering and pollination efficiencies were negatively and significantly correlated within nine of the ten crosses (Table 1). A negative correlation was also obtained between the number of flowers and the number of pods harvested six months later. However, it was significant only in six crosses and with lower r values.

Cherelle Wilt

An average of 263.8 cherelles per tree were counted in the ten SG2 hybrid crosses (Table 3). An average of only 73.4 (27.9%) remained green. The others (72.1%) were removed as either yellow or black cherelles.

Table 3. Average number and proportion (%) of yellow, black and green cherelles/tree of the ten SG2 hybrid crosses in 1999 and 2000

Year	Number of cherelles/tree			Total	Proportion (%)		
	Yellow	Black	Green		Yellow	Black	Green
1999	38.0	137.0	69.3	244.3	15.6	56.1	28.4
2000	42.6	163.3	77.4	283.3	15.0	57.6	27.3
Average	40.3	150.2	73.4	263.8	15.3	56.8	27.9

The number of black cherelles was 3.7 times higher than the number of yellow ones (physiological wilt?). The results in 1999 and 2000 were very similar.

The ten crosses produced different average numbers of cherelles per tree (Table 4), ranging from 158 (KEE 42 x KA2-106) to 395 (KEE 47 x KA2-106). Despite the negative correlation between the number of flowers and pollination efficiency, there were more cherelles produced in the crosses that had higher numbers of flowers. The two variables, number of flowers and number of cherelles were highly significantly correlated (r = 0.77) for all the trees of the ten crosses. The rankings of the crosses for the number of cherelles were similar in 1999 and 2000, except for the cross KEE 12 x KA2-106. The proportions of green cherelles, however, were similar in most of the crosses, unrelated to the total number of cherelles produced except for KEE 42 x KA2-106, which had the highest proportion of green cherelles in both 1999 and 2000.

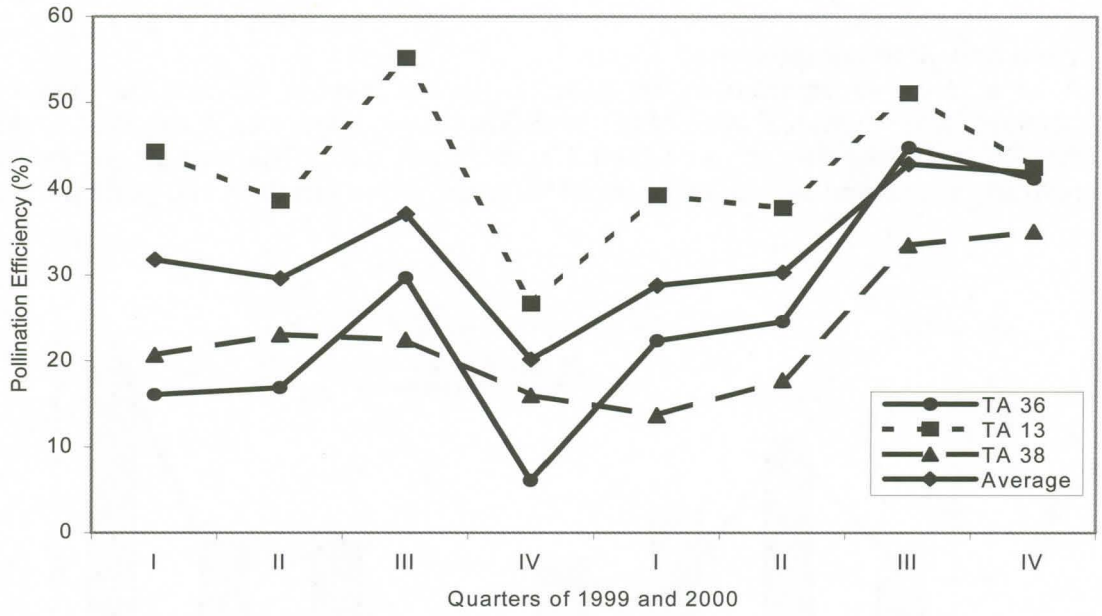

Figure 2. Average quarterly pollination efficiencies of the SG2 hybrids and three selected crosses during 1999 and 2000

The ranking of the crosses in the two years was not similar.

The proportions of green cherelles and accordingly the wilted cherelles varied throughout the year and were very closely associated with the amount of rainfall (Figure 3). The highest proportions of green cherelles were obtained in the second quarter of 1999 and the fourth quarter of 2000, the periods with the highest rainfall. Similarly, the lowest proportion of green cherelles was obtained in the first quarter of 2000, the period with the lowest amount of rain. The other periods followed a similar pattern . This pattern was similar in all ten crosses.

Table 4. Average number of cherelles/tree and the proportion of green cherelles for ten SG2 hybrid crosses during 1999 and 2000

TA cross no.	Pedigree	Av. no. of cherelles/tree			Green cherelles (%)		
		1999	2000	Average	1999	2000	Average
38	KEE 47 x KA2-106	475 (1)	314 (2)	395	27 (9)	30 (2)	28.5
13	KEE 12 x K82	318 (2)	322 (1)	320	28 (7)	23 (10)	25.5
31	KEE 5 x KA2-106	264 (4)	314 (3)	289	31 (3)	28 (5)	29.5
33	KEE 12 x KA2-106	316 (3)	252 (8)	284	27 (10)	27 (6)	27.0
35	KEE 23 x KA2-106	234 (5)	304 (4)	269	31 (4)	26 (7)	28.5
37	KEE 43 x KA2-106	192 (7)	294 (5)	243	30 (5)	24 (9)	27.0
17	KEE 43 x K82	201 (6)	275 (6)	238	29 (6)	29 (3)	29.0
11	KEE 5 x K82	168 (8)	249 (9)	209	28 (8)	29 (4)	29.5
16	KEE 42 x K82	104 (9)	254 (7)	179	32 (2)	26 (8)	29.0
36	KEE 42 x KA2-106	87 (10)	229 (10)	158	37 (1)	34 (1)	35.5

( ) The numbers in brackets represent the rank

Yield and yield components

A wide range of variation in the average annual number of pods per tree was obtained in the individual trees of the ten SG2 crosses (Figure 4). It ranged from less than 20 pods per tree to more than 130 pods per tree. The pod production was normally distributed with a tail of about 20 trees with more than 100 pods per tree.

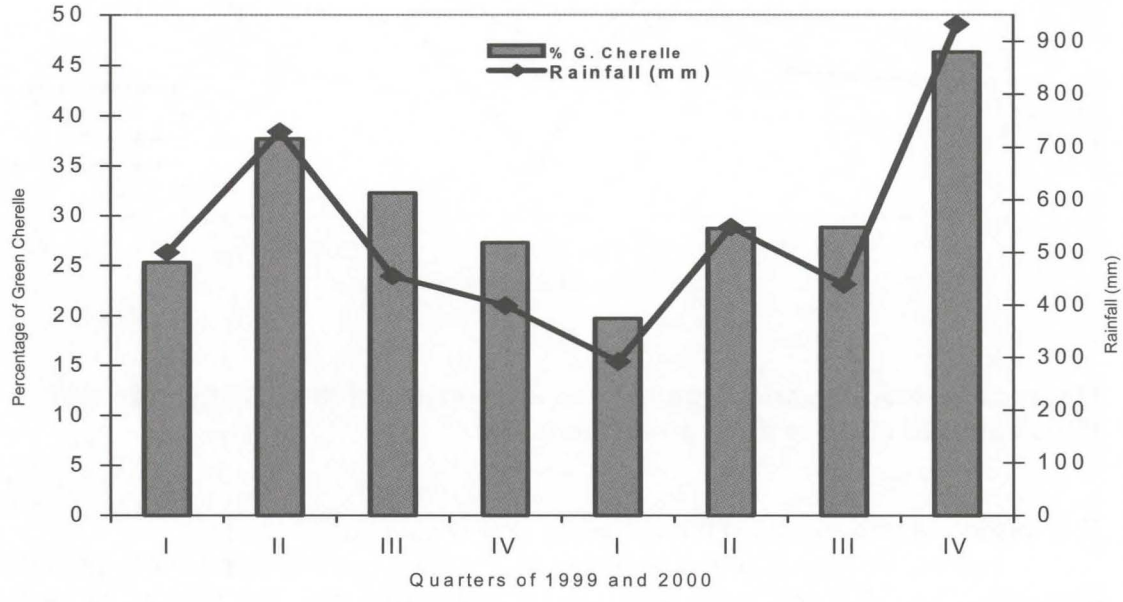

Figure 3. An average quarterly proportion (%) of green cherelles and the amount of rainfall during 1999 and 2000

Yield per hectare was estimated from the average number of pods/tree/cross that ranged between 49.0 (KEE 23 x KA2-106) and 74.8 (KEE 12 x K82), the average pod weight (292 g for KEE 12 x K82 to 588 g for KEE 5 x KA2-106) and the percentage of wet beans in the pod (25.6% for KEE 47 x KA2-106 to 33.6% for KEE 5 x KA2-106). The average annual dry bean yield of the ten crosses varied between 1017 (KEE 12 x KA2-106) and 1684 (KEE 23 x KA2-106) kg ha^{-1} (Table 5). The average yield in 1999 was higher than in 2000, but the differences between the crosses were consistent (similar ranking).

The two Trinitario clones were similar as donors for yield and yield components (Table 6), but the Upper Amazonian parents were different. KEE 42 was a donor for low number of big pods, KEE 5 was a donor for intermediate sized pods with the highest percentage of wet beans and KEE 12 was a donor for the highest number of the smallest pods and the lowest percentage of wet beans.

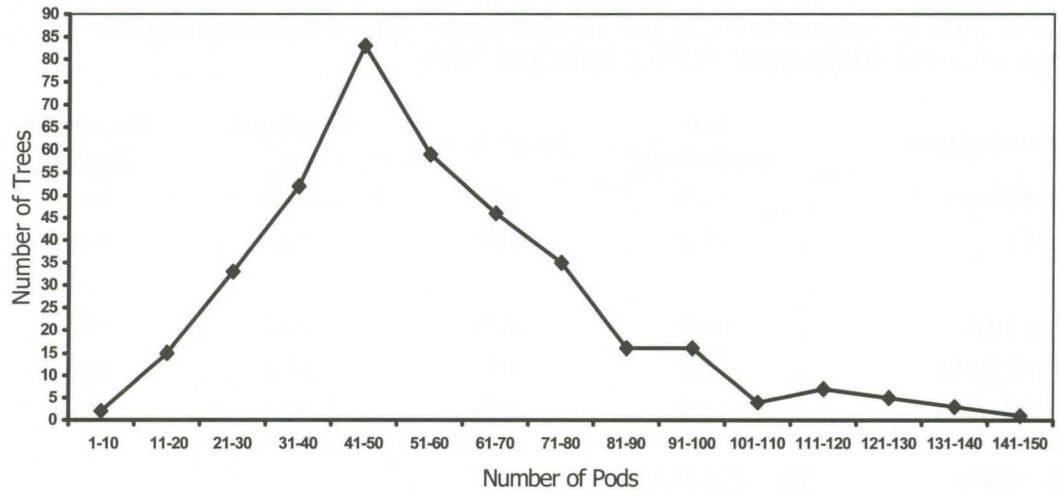

Figure 4. Frequency distribution of the average annual number of pods per tree of 377 trees derived from ten SG2 crosses during 1999 and 2000

Comparison between high and low yielding trees

A comparison between high and low producing trees can provide a possible explanation for the variability in the production of individual trees. Averages of 95.5 pods/tree were harvested from the 40 highest yielding trees and 28.1 pods from the 40 lowest yielding trees (Table 7). The proportional difference [(1 – low:high) x 100] was +70.6%. Neither the pollination efficiency, nor the proportion of green cherelles could account for the difference between the high and low yielding trees. The high yielding trees had more flowers than the low yielding trees, but this was partially offset by their lower pollination efficiency and the lower proportion of green cherelles.

Table 5. Average annual yields and yield component of ten SG2 crosses during 1999 and 2000

TA cross no.	Pedigree	No. pods/tree	Pod wt. (g)	Wet bean (%)	Dry bean (kg/ha)
31	KEE 5 x KA2-106	69.4 (1,2)	385	33.6	1684
11	KEE 5 x K82	64.3 (4,4)	421	31.5	1599
37	KEE 43 x KA2-106	62.9 (5,3)	449	31.1	1647
33	KEE 12 x KA2- 106	64.8 (3,5)	303	27.6	1017
13	KEE 12 x K82	74.8 (2,1)	292	28.3	1159
36	KEE 42 x KA2-106	50.3 (9,6)	588	28.5	1579
16	KEE 42 x K82	49.3 (7,10)	551	29.2	1487
17	KEE 43 x K82	55.1 (6,7)	420	27.4	1190
35	KEE 23 x KA2-106	49.0 (10,9)	428	28.5	1120
38	KEE 47 x KA2-106	50.2 (8,8)	424	25.6	1022

( ) The number in brackets represents the rank in 1999 and 2000, respectively.

However, the average number of cherelles per cross was a function of both the number of flowers and pollination efficiency. As a result, the crosses with the higher number of flowers produced more cherelles than crosses with lower numbers of flowers ($r = 0.77^{**}$). The negative correlation within the crosses was partially maintained to the stage of mature pods, but at a lower magnitude and only in six of the crosses. This was probably due to the effect of cherelle wilt and pod losses. At the end, there were no apparent relationships between crosses for the average number of flowers per cross and the number of pods harvested per cross six months later. Thus, the highest and lowest flowering crosses produced similarly low numbers of pods. At the same time, the highest number of pods was harvested from a cross with an intermediate number of flowers.

The pollination efficiencies varied between and within crosses. They were negatively correlated with the number of flowers. Given that cocoa is pollinated by insects, there were probably insufficient numbers of pollinating insects to pollinate all the flowers. The differences between crosses, their similar ranking in 1999 and 2000, and the apparent parental effect showed that pollination efficiency was under genetic control. However, since the pollination efficiencies were negatively correlated with the number of flowers, which was also under genetic control, it is possible that the same genes that control the number of flowers are affecting, at least partly, the pollination efficiency. Namely, the primary effect of the genes is to determine the number of flowers, and thus indirectly also pollination efficiency due to the limited number of pollinating insects. There were also some indications of additional genetic differences that were unrelated to the number of flowers. These may be due to differences in the attractiveness of the flowers to pollinating insects.

Cherelle wilt is a major limiting factor for cocoa production. About 70% of the cherelles produced (3-5 cm) wilted during 1999 and 2000. The wilting was not uniform throughout the year. It was closely associated with the rainfall such that as the rainfall increased, the proportion of wilted cherelles decreased. Preliminary work at CCRI (Saul, Konam and Namaliu, unpublished) showed that *Colletotrichum* sp. was associated with the wilting cherelles. A similar association was reported by Dakwa (1978) in Ghana. Usually, there were no visible signs of moisture stress on the cocoa trees. Even during the dryer period, the average monthly rainfall was about 100 mm. *Colletotrichum* is usually an endophyte or a weak pathogen in cocoa (Blaha, personal communication). It is possible that in dryer conditions the cherelles became more vulnerable to *Colletotrichum* attack. However, the possibility that the cherelles wilted physiologically first at higher proportions in dryer conditions and were then invaded by *Colletotrichum* was not ruled out. Usually, there were no genetic differences between nine of the ten SG2 crosses. Only one cross (KEE 42 x KA2-106) had a somewhat higher proportion of unwilted green cherelles. The absence of genetic variability and the uniform effect of the environment may explain the similar proportions of green cherelles in high and low yielding trees. If *Colletotrichum* is verified to be a cause of cherelle wilt, emphasis should be given to search for sources of resistance and breeding. The development of efficient screening methodologies is a prerequisite for a successful breeding programme.

Losses continued to occur later during pod development. These losses accounted for most of the difference between the high and low yielding trees. Unfortunately, the period beyond the cherelle stage of 3-5 cm long was not followed closely. Therefore, there were no indications of the causes and timing of the losses. However, they were most probably caused by late cherelle wilt, diseases or both. The difference between high and low yielding trees suggested that there were genetic differences between trees. Wood and Lass (1985) indicated that only 1-5 percent of the flowers are successfully pollinated to produce pods. This value (4.1%) was obtained for the group of low yielding trees. However, a higher average value of 10.4% was obtained for the group of high yielding trees. This showed that higher yielding trees were more efficient in converting the flowers into pods. A better

understanding of the mechanisms involved would assist the breeder in developing higher yielding cocoa genotypes.

The number of pods per tree was only one of the yield components. Pod weight and the percentage of wet beans in the pods were also important. Genetic differences were found between the SG2 crosses and the parental clones for these two components. Breeding and selection for desirable pod weight and percentage of wet beans combined with higher numbers of pods is therefore possible in order to increase yield potential.

Conclusion

Cocoa pod production is a long and complex process of several stages, each controlled by a different set of polygenes and affected by the environment. The different stages may or may not be related to each other and may be affected differently by the environment.

Genetic variability was identified between and within the ten SG2 crosses for most of the variables tested. The genetic variability within crosses is probably due to the heterozygosity of the parental clones and genetic segregation in the progenies of individual crosses. Thus, each cross represents a population of trees with distinguishable means for various characteristics. An improvement of the parental clones by accumulating desirable genes can shift the population mean in the desired direction. This can be done by various population improvement schemes. However, it is a very long term and laborious process.

Cocoa can be reproduced vegetatively. The development of high yielding cocoa clones will eliminate the problem of genetic variability because all the trees of the same clone, though heterozygous, are genetically identical. Therefore, the development of clones is a preferred breeding approach.

Acknowledgement

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DK-52767g.

Comparison of Values as Clones and as Progenitors for Yield, Vigour and Yield Efficiency: Experiences from Côte d'Ivoire

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Abstract

The study presented in this paper consisted of three comparisons between the performances of 23 clones evaluated in five clonal trials and their performances as progenitors in three progeny trials in Côte d'Ivoire, for yield, vigour and yield efficiency. Some significant correlations were observed in the case of two of the three progeny trials, but the sense of the correlations changed according to the genotypes under study, making the results inconclusive. Additional studies should be carried out, with much larger numbers of genotypes and in different environments, in order to obtain a clear appreciation of the possible use of clone values to predict combining ability.

Introduction

Knowledge of heritability of traits of interest is very important in choosing the best breeding strategy. In cocoa breeding, several authors evaluated the heritability of yield, using diallel or factorial designs and obtained rather contrasting values for both broad and narrow sense heritabilities. These variations in heritability values are in a large part caused by different ways of calculation (*e.g.* fixed *versus* random effects, different replicate numbers, and different plot sizes).

Lockwood and Pang (1994) showed the predominance of General Combining Ability (GCA) for early yield by comparing the values observed for progenies and the values calculated using the GCAs of the parents of these progenies. These authors also compared the values of clones in clonal trials with their GCAs in progeny trials, for early yield. They always found positive rank correlations, but this correlation was significant only in the case of one of the six pairs of compared trials (Lockwood and Pang 1993).

The same type of comparison was performed in Côte d'Ivoire. Twenty-three clones were evaluated as clones in five clonal trials and as progenitors in three progeny trials in Côte d'Ivoire, for yield, vigour and yield efficiency.

Material and methods

The clone and progeny trials are indicated in Tables 1 and 2. A local Amelonado clone, IFC 5, is a control in all the clone trials and is used to allow the comparison between clones present in different clone trials, by dividing their value by the one obtained for IFC 5.The genotypes tested as clones in the clone trials and as female progenitors in progeny trials are as follows:

- Genotypes tested as clones in the clone trials (indicated in brackets) and as female progenitors in the A19/1 hybrid trial are T 79/501 (D11/2), PA 7 (G8),

POUND 7 (D13/4), T 60/887 (D13/4), NA 32 (D11/2), SCA 6 (G8), T 63/967 (D13/4) and PA 150 (G8).

- Genotypes tested as male progenitors in A19/1 hybrid trial: IFC 1, IFC 2, IFC 5 and IFC 15
- Genotypes tested as clones in the clone trials (indicated in brackets) and as female progenitors in E4/1 hybrid trial *(9 x 4 NC II design)*: AMAZ 15/15 (C2/2), EQX 94 (C2/2), IMC 6 (A18/2), IMC 67 (C2/2), MO 81 (A18/2), MO 98 (C2/2), NA 32 (D11/2), POUND 19/A (A18/2) and UPA 401 (D11/2)
- Genotypes tested as male progenitors in E4/1 hybrid trial (9 x 4 NCII design): IMC 78, POR, T 60/887 and UPA 413
- Genotypes tested as clones in the clone trials indicated in brackets and as female progenitors in E4/1 hybrid trial (8 x 5 NC II design): the same as for 9x 4 NCII design, except for the absence of MO 81
- Genotypes tested as male progenitors in E4/1 hybrid trial (8 x 5 NCII design): the same as for 9 x 4 NCII design, with the addition of SCA 6
- Genotypes tested as clones in the clone trials indicated in brackets and as female progenitors in E4/1 hybrid trial (4 x 7 NC II design): PA 150 (G8), SCA 6 (G8), T 60/887 (D13/4) and UPA 413 (D13/4)
- Genotypes tested as male progenitors in E4/1 hybrid trial (4 x 7 NCII design): AMAZ 15/15, EQX 94, IMC 6, NA 32, POUND 19/A, UPA 401 and UPA 409
- Genotypes tested as clones in the clone trials indicated in brackets and as female progenitors in C2/1 hybrid trial: IMC 67 (C2/2), PA 150 (G8), ICS 39 (D11/2), ICS 89 (D11/2), POUND 19/A (A18/2), PA 13 (A18/2), PA 121(A18/2)
- Genotypes tested as male progenitors in C2/1 hybrid trial: SIC 864, SPEC 160/9, IMC 67 and PA 150

Results and discussion

Table 3 shows the significant correlations observed between relative values of the clones in the clone trials and the values of their progenies in hybrid trials for the traits under evaluation.

In the case of A19/1 progeny trial, significant positive correlations are observed between the yield of the clones in the clone trials and the canopy surface of their progenies in the hybrid trial (r = 0.83), and between the trunk circumference of the clones and that of the progenies (r = 0.72). These positive correlations and the absence of correlation between the yield of the clones and the yield of the progenies can explain the negative significant correlations observed between the yield of the clones and the yield efficiency of the progenies, and between the vigour of the clones and the yield efficiency of the progenies.

In the case of E4/1, significant positive correlations are observed between the yield value of the clones in the clone trials and the trunk circumference of the progenies in the hybrid trial, for both 9x4 (r = 0.66 and r = 0.73 for Pearson and Spearman coefficients, respectively) and 8x5 (r = 0.7 and r = 0.76 for Pearson and Spearman coefficients, respectively) NCII designs. In the case of the 4x7 NCII design, significant negative correlations are observed between the yield value of the clones in the clone trials and the yield, vigour and yield efficiency values of their progenies in the progeny trial (r = -0.98, -0.95 and – 0.99 respectively). This difference can be attributed to the fact that completely different genotypes were studied here, particularly with the inclusion of SCA 6, which shows strikingly different performances as a clone and as progenitor.

In the case of the C2/1 progeny trial, no significant correlation is observed.

Table 1. Details of the clone trials

Clone trial	Year of planting	Locality	Harvest campaigns	Vigour measurement	Statistical design	Number of replications
D 11/2	1971	Divo	79/80 to 81/82	Trunk circumference 1991	Fisher blocks	4 rows of 12 trees
D 13/4	1972	Divo	77/78 to 81/82	Trunk circumference 1991	Fisher blocks	4 rows of 12 trees
G 8	1973	Divo	78/79 to 81/82	Trunk circumference 1991	Fisher blocks	4 rows of 12 trees
C 2/2	1990	Bingerville	92/93 to 95/96		Single tree randomisation	20 trees
A 18/2	1983	Divo	87/88 to 91/92		Single tree randomisation	62 trees

Table 2. Details of the progeny trials

Progeny trial	Year of planting	Locality	Statistical design	Number of replications
A 19/1	1979	Divo	Single tree randomisation	17 trees
E 4/1	1992	Divo	Single tree randomisation	20 trees
C 2/1	1988	Bingerville	Single tree randomisation	40 trees

Conclusion

Even if some significant correlations were observed in our studies, the values of these correlations vary largely between the trials and, consequently between the genotypes evaluated as clones and as progenitors. Lockwood and Pang (1993) suggest that much higher correlations could be obtained if clones and progenies are evaluated at their optimal planting densities. Optimal planting densities have shown to vary to a great extent with the clones under evaluation (Mooleedhar and Lauckner 1990; Lockwood and Pang 1996). In our case, all the clones and all the progenies were evaluated at a single planting density. On the other hand, we would not exclude other types of interactions, such as canopy shape, which may affect early yield more for clones than for seedling progenies.

Table 3. Significant correlations observed between the relative values of the clones in clone trial and the values of their progenies in hybrid trials

Trial	Trait observed on the clones	Trait observed on the progenies	Type of correlation	r	p
A19/1	Yield	Vigour (canopy surface 89)	Spearman	0.83	0.01
A19/1	Vigour	Vigour (trunk circumference 94)	Spearman	0.72	0.04
A19/1	Yield	yield efficiency (yield 83-88 / canopy surface 89)	Spearman	-0.86	0.006
A19/1	Vigour	yield efficiency (yield 83-88/trunk circumference 89)	Pearson Spearman	-0.7 -0.78	0.05 0.02
A19/1	Vigour	yield efficiency (yield 83-88 / trunk circumference 94)	Spearman	-0.75	0.03
E4/1 (9x4 NCII)	Yield	Vigour (trunk circumference 98)	Pearson Spearman	0.66 0.73	0.05 0.03
E4/1 (8x5 NCII)	Yield	Vigour (trunk circumference 98)	Pearson Spearman	0.7 0.76	0.05 0.03
E4/1 (4x7 NCII)	Yield	Yield (Yield 98-2000)	Pearson	-0.98	0.02
E4/1 (4x7 NCII)	Yield	Vigour (trunk circumference 98)	Pearson	-0.95	0.05
E4/1 (4x7 NCII)	Yield	Yield efficiency (Yield 98-2000 / trunk circumference 98)	Pearson	-0.99	0.01

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Individual Tree Variation and Selection for Yield and Vigour: Experience from Côte d'Ivoire

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Abstract

This study entailed a comparison of the performances of 22 single trees selected in a progeny trial in Bingerville (south-eastern Côte d'Ivoire) with those of the clones issued from their vegetative multiplication (plagiotropic cuttings), evaluated in a clonal trial at the same locality. The traits observed were early and adult yield, vigour and yield efficiency. When compared to a seedling control progeny present in both trials, the selected genotypes showed a much lower relative early yield value in the clone trial than in the hybrid trial. This might suggest superiority for early yield of seedling trees in comparison to plagiotropic clonal trees. In addition, a similar level of variation was observed between trees within a seedling progeny and within a clone, suggesting that inter-tree variation is mainly due to environment.
Correlation analysis revealed that the values of clones could not properly be predicted from those of the single trees from which they were obtained. On the other hand, the average values of the progenies to which the selected trees belong appeared as better predictors of the values of the clones obtained from these progenies, but the correlations remained rather low (r values of roughly 0.5), even when significant, and only the yield assessed on the trees between their tenth and fifteenth year was a significant predictor. These results seem to indicate a rather poor efficiency in the selection of individual trees for obtaining clones with high early yield. Possibly, data from additional years of harvesting of the clones are necessary before reaching final conclusions on the selection efficiency of individual trees based on yield.
These results are in contrast to the significant correlation between individual tree values and clone values for *Phytophthora* pod rot incidence in the same trials, as published earlier. Under the conditions of these trials, pod rot incidence seems therefore to be a more heritable trait than yield and vigour. An individual tree selection strategy based on combined selection for resistance to black pod and yield is discussed.

Introduction

In Côte d'Ivoire, cocoa breeding programmes have been based on the creation of progenies issued from crosses between progenitors of several genetic origins (Upper Amazon Forastero, Amelonado, Trinitario) (Besse 1977; Paulin and Eskes 1994). These progenies have been distributed as pods issued from bi-clonal seed-gardens. Observations in the progeny trials have shown a rather high level of variation among trees issued from the same cross, and two ways of creating more homogeneous progenies were considered. One method involved the use of doubled haploids (DH) (Dublin 1978), but this approach has been restricted to clones of very few genetic origins that provided spontaneous DH (Falque 1994). Moreover, trials using bi-parental crosses involving two DH failed to show a consistently improved homogeneity of this type of genetically uniform progeny compared to classical full-sib progenies between heterozygous parents (Sounigo *et al.* 2003). An alternative approach involves the use of clonal varieties. This approach seemed particularly promising because it was envisaged that it would enable us to create homogeneous varieties issued from the best trees selected in our progeny trials.

In order to test the validity of the second strategy, 22 high yielding trees were selected in a progeny trial and were vegetatively propagated as plagiotropic cuttings before being planted in a clonal trial. The yield, vigour and yield efficiency of these clones in the clonal trial were compared to those of the progenitor in the progeny trial. Comparisons were made in relation to a control seedling progeny (IMC 67 x IFC 1) planted both in the original hybrid trial and in the clone trial. This comparison was performed in order to assess the efficiency of our clone selection for reproducing the performances of individual trees and improving the homogeneity of the selected varieties.

Material and methods

Full-sib progeny trial

Planted in 1979, this trial has been used to compare 57 full-sib progenies, according to an incomplete factorial design (crosses between 16 Upper Amazon Forastero x 4 Amelonado genotypes). Each full-sib progeny is represented by 18 trees, according to a randomised single-tree design (Lotodé 1971). The planting density adopted was 1,333 trees ha^{-1}, and the cocoa plants were planted under temporary shade provided by *Glyricidia maculata* trees during the first two years.

Individual tree observations conducted in the full-sib progeny trial

Juvenile vigour was measured by the increase in trunk diameter between one and two years after planting. Adult vigour was assessed by measuring the trunk circumference in 1989 and in 1994 and the canopy surface in 1989. The yield was measured according to the method proposed by Lachenaud (1984), consisting of weighing the harvested pods and converting the pod weight into fresh bean weights at each harvest (approximately ten harvests per year). These measurements were performed during the periods from 1983 to 1987 (four harvest campaigns) and from 1990 to 1995 (five harvest campaigns).

Selection of individual trees in the progeny trial

The 22 trees were selected in 1987 based on observations made during the period from 1983 to 1987. They were vegetatively propagated by the use of plagiotropic cuttings.

Clone trial

Planted in 1987, this trial has been used to compare the 22 clones issued from the 22 trees selected in the full-sib progeny trial. Each clone is represented by 10 to 21 trees, corresponding to 10 to 21 replications, according to an unbalanced randomised single-tree design (Lotodé 1971). In addition, seedlings from the crosses IMC 67 x IFC 1 were also planted as controls, thus allowing the comparison between the values of the clones in the clonal trial and the values of the original trees in the full-sib progeny trial. The planting density adopted was 1,333 trees ha^{-1}, and the cocoa plants were planted under temporary shade provided by plantain during the first two years.

Individual tree observations conducted in the clone trial

The vigour was measured by the trunk section of the trees in 1990. The yield was measured in the same way as in the full-sib progeny trial. These measurements were performed during the periods from 1989 to 1994 (five harvest campaigns).

Results and discussion

Homogeneity for yield within the full-sib progenies and within the clones

The coefficients of variation (C.V.) observed for yield during the period from 1983 to 1987 within the progenies in the full-sib progeny trial vary between 26% and 95%, with a mean value of 62%, the value for the control (IMC 67 x IFC 1) being 87%. Those observed in the clone trial vary between 19% and 55%, with a mean value of 35%, the value for the control (seedlings from IMC 67 x IFC 1) being 36%.

This result shows a lower level of variation in the clone trial than in the full-sib progeny trial. However, in the clone trial, the control, which is a full-sib progeny, shows a C.V. comparable to those of most of the clones. This indicates that the differences in homogeneity between the two trials cannot be attributed to the higher genetic homogeneity within the clones than within the full-sib progenies, but, most probably to a better environmental homogeneity in the clone trial plot and possibly also to less competition between trees in the younger clone trial. This result confirms the lack of clear evidence of a consistently higher homogeneity within the progenies issued from crosses between doubled haploids compared to full-sib progenies (Sounigo *et al.* 2003). This indicates that, in our trials with a single-tree randomisation design, the major part of the variation within a full-sib progeny is due to environment rather than to genotypic differences between trees belonging to the same full-sib progeny. The same level of homogeneity within progenies and in clones was also observed for *Coffea canephora* (Montagnon 2000).

Comparison of yield of progenies, selected trees and clones

Table 1 and Figures 1 and 2 compare the relative yield (in % of the yield of the control) of entire progenies and of selected trees in the progeny trial (yield during the period from 1983 to 1987) with that of the yield of clones in the clone trial (1990 to 1994).

They show that:

- All the clones show a lower relative yield value than the selected trees from which they were obtained. The relative difference between a clone and its tree of origin varies between 31% and 186%.
- Only seven of the clones show a higher relative yield value than their progeny of origin, the differences varying between 14% and 31%. These seven clones come from the lowest yielding progenies. The rest of the clones show a lower relative yield value than their progenies of origin, the differences varying between 3% and 84%. These clones come from the highest yielding progenies and the highest differences are observed for the highest yielding progenies. In addition, the three best progenies show a higher early yield than the best clone.

These results show that the relative values of the clones for early yield are rather disappointing, compared to the ones observed on the selected trees (Figure 1) and to the better progenies from which they were derived (Figure 2). This raises doubts about the advantages of clonal selection for this trait. However, the low relative early yields of the clones could be partly due to the relative advantage of the seedling plant stature of the control progeny in the clone trial (orthotropic growth, more exposed to light than the rooted cuttings at young age).

Correlations between selected trees and clones

Coefficients of correlation were calculated between the yield, vigour and yield efficiency values of individual trees in hybrid trials and the values for the same traits in the clones issued from these individual trees, in the clone trial. No significant correlation was observed for yield or yield efficiency. Only one significant negative correlation was observed between the vigour of the selected tree (relative trunk circumference increase between 1989 and 1994) and of the clone (trunk diameter measured in 1989) but its value was low (- 0.45).

In addition, correlations were calculated between values observed for different traits (yield of the individual tree *versus* (vs) vigour of the clone, yield of the individual tree vs yield efficiency of the clone, vigour of the individual tree vs yield of the clone, vigour of the individual tree vs yield efficiency of the clone, yield efficiency of the individual tree vs yield of the clone, yield efficiency vs vigour of the clone) but none of them was significant.

The lack of correlation between the values of the selected trees and those of the clones highlights the difficulty in selecting individual trees as mother trees for clone selection. This is not surprising if most of the variation between trees within the same progeny is due to the environment rather than to genotypic differences. Another reason to explain this lack of correlation could be the fact that the clones are represented by cuttings. Indeed, in most cases, cuttings do not have taproots and it is possible that this attribute induces differences for early yield with the seedlings from which they are issued. Such differences could be due also to different canopy shapes of the clones (more or less erect branching), which may affect early yield more for clones than for seedling trees.

Table 1. Comparison between the yield observed in the progeny and in the clone trials

	Progeny trial	**Clone trial**
	Yield 83/84 - 86/87[1]	**Yield 89/90 - 93/94[1]**
Mean value of IMC 67 x IFC 1	15.1	14.5
Mean value of the trial	12.3 (81%)	13.2 (91%)
Range of values of the progenies	2.4 (16%) - 24.8 (164%)	
Range of values of the selected trees	20.7 (137%)- 42.1 (279%)	
Range of values of the clones		7.5 (51%) - 18.5 (128%)

[1]The values correspond to grams of fresh beans, except those in brackets, which are expressed as % of the control (full-sib from the cross IMC 67 x IFC 1)

Correlations between mean of progenies and clones derived from the same progenies

Correlations were calculated between the values obtained for the progenies in the hybrid trial and those for the clones issued from individual trees of these progenies, in the clone trial. The traits observed were yield, vigour and yield efficiency and correlations were calculated between values obtained for the same traits and between values obtained for two different traits.

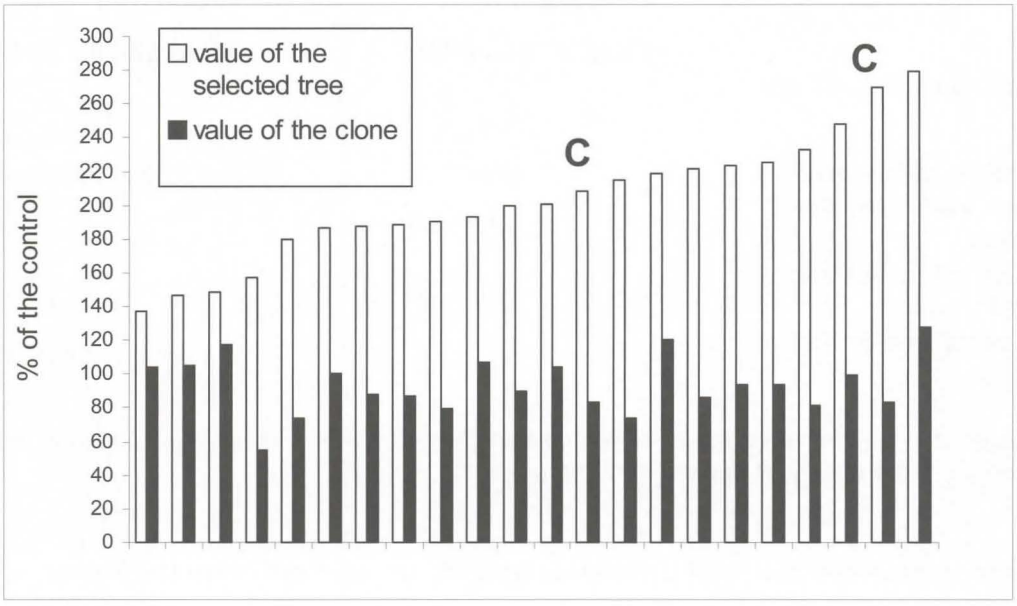
Table 2 shows a low but significant positive correlation (r= 0.47) between the yield of the progeny from which the tree was selected and the yield of the clone obtained from the selected trees only in the case when yield was recorded during the period from 1990 to 1994. It also shows significant positive correlations between the yield efficiency of the progenies and the yield of the clones when the former is measured as the ratio between yield during the period 1990 to 1994 and the juvenile vigour (diameter increase at the level of the collar during the second year after planting) (r = 0.56) and when it is measured as the ratio between the relative yield increase and the relative vigour increase (r= 0.46).

These results show that the selection of individual trees can be improved by selecting the trees within the highest yielding progenies and the progenies with the highest yield efficiency, on the condition that these traits are not measured on young trees. In our case, yield measurements were better indicators when they were performed between the 11th and the 15th year.

Conclusion

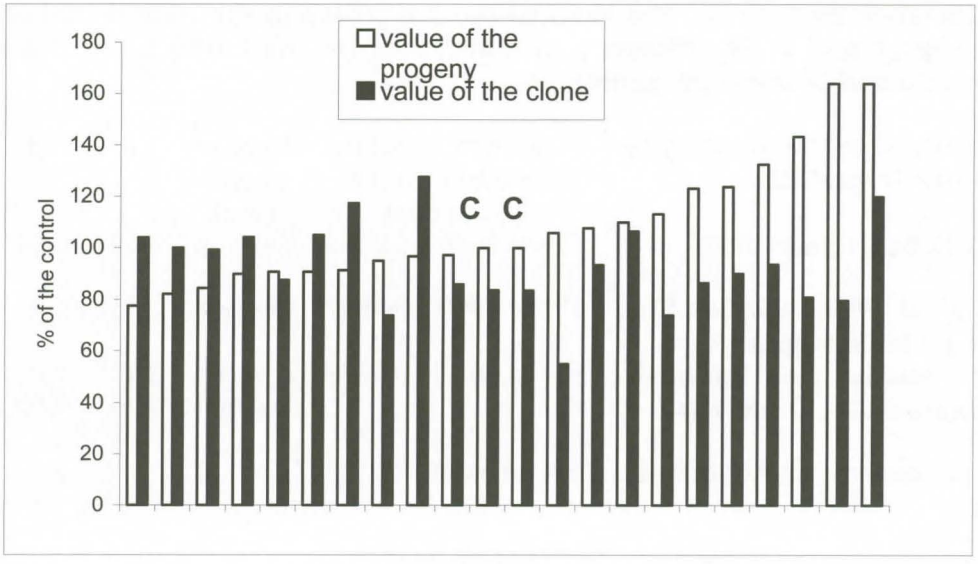
The data obtained in this study raise doubts about the advantages of clonal selection for obtaining homogeneous varieties with a good early yield. They also show the difficulty of efficiently selecting individual trees for yield, vigour and yield efficiency.

Since correlations where improved when the values of the clones were compared to those of the progenies from which the trees were selected, a possibility of improving individual selection seems to exist by selecting the trees only within the best progenies, which would ensure that the good performances of the trees are not only the results of favourable environmental conditions.



C = Control progeny

Figure 1. Comparison of the relative early yield of the selected trees (1983 to 1987) in the progeny trial and that of the clones in the clone trial (1990 to 1994)



C = Control progeny

Figure 2. Comparison of the relative average early yield of the entire cross-progenies (1983 to 1987) in the progeny trial with that of the clones in the clone trial (1990 to 1994)

We only observed the clones for early yield, and therefore recommend that some additional measurements of yield be performed in the same clone trial now that the trees are much older (more than 15 years-old), in order to evaluate the efficiency of the clonal selection for yield of adult trees. In addition, it would be useful to make the same type of comparison, using clones propagated as grafted or budded plants, in order to ensure that the use of cuttings issued from plagiotropic branches is not a serious handicap. Another limitation for the selection of individual trees is the single tree randomisation design, in which the influence of environmental variations is maximised at the level of each tree. The performances of the trees are often modified by the vigour and the "aggressiveness" of their neighbouring trees (Lachenaud and Montagnon 2002), especially with the planting density adopted in our trials, which is too high to allow every tree to develop without any competition with its neighbours (Lachenaud and Oliver 1998). It is then possible that individual tree selection would be easier in designs with replication units composed of several trees. On the other hand, one advantage of the single-tree randomisation design is that it is most likely to correspond to the situation found in many cocoa farms, where cocoa farmers mix the seeds obtained from their pods before sowing them. Hence, if an efficient method of individual tree selection can be identified in a research station, it will be more applicable for tree selection in cocoa farms. In addition, the use of correcting coefficients can be made in order to reduce the impact of the neighbouring trees on the measurements (Cilas, 2003).

Table 2. Correlations between the yield of the progenies in the hybrid trial and the yield, vigour and yield efficiency of the clones (in the clone trial), issued from trees selected in these progenies

Trait observed in the progeny of the hybrid trial	Trait observed in the clone in the clone trial	Type of coeffi-cient	r	p
Yield 90/91 and 94/95	Yield 90/91 - 94/95	Pearson	0.47	0.04
Ratio of yield (90-91 and 94-95) to juvenile vigour	Yield 90/91 - 94/95	Pearson	0.56	0.01
Ratio of the relative yield increase to the relative vigour increase	Yield 90/91 - 94/95	Pearson	0.46	0.05
		Spearman	0.46	0.05

r = value of the coefficient of correlation and p = probability of r
Juvenile vigour = increase in the collar diameter during the period from the first to the second year after planting
Relative yield increase = (yield 1990-94 / yield 1983-87) *100
Relative vigour increase = ((trunk circumference 1994 – trunk circumference 1989) : trunk circumference 1989)) x 100

Even if further studies confirm the poor efficiency also for individual tree selection of older trees, this does not mean that clonal selection should not be applied, since it seems to be promising for improving resistance to *Phytophthora.* Indeed, Kébé *et al.* (1999) found values ranging between 0.6 and 0.7 for the coefficients of correlations obtained between the percentage of rotten pods recorded on the selected individual trees and those recorded for the corresponding clones.

Two different strategies could be adopted, depending on the data available:

- When working on new progenies, the best method would probably be to use early screening tests for *Phytophthora* resistance (leaf test and/or a pod inoculation test on young trees). The most resistant plants would then be cloned and evaluated in observation plots or trials, for resistance and yield. This strategy would need to include a large number of clones, to ensure that enough genetic variation is present in the selection plots to be able to select effectively for yield.
- The most efficient strategy would probably be to use data available from old trials on yield and black pod incidence. Within the highest yielding progenies the most resistant trees (based on field observations and early resistance screening methods) could be selected for cloning. These selected trees should then be vegetatively propagated and assessed for their yield, yield efficiency and resistance to *Phytophthora* in clone trials and the best clones could be identified based on index selection for these traits. This strategy should be more efficient for selecting for both resistance and yield than the previous one, but requires the availability of field data on the seedling progenies, particularly for adult yield.

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Correlation Between Clone Values in the Germplasm Collection and Hybrids Produced by these Clones at CEPEC, with Special Emphasis on the Yield x Vigour Relationship

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Abstract

The prediction of the general combining ability (GCA), from the performance of accessions *per se* in germplasm collections, and the methodologies of analyses that can improve the efficiency of evaluation, are important points to be investigated in the optimisation of breeding programmes. Aiming to understand these aspects, data from 544 accessions in CEPEC's germplasm collection were analysed and compared with GCA values calculated from eight progeny trials (one diallel and seven NCII designs). The accessions were each represented by one row of 10 trees, except in the case of 21 accessions that were represented by two rows, each of 10 trees. The following variables were observed for mature trees over six years in the collection, and over one to eight years in the hybrid trials: wet bean weight per tree (WBW), number of healthy pods (NHP) and trunk sectional area (TS). Parent-offspring correlations were calculated based on the clonal performance in the collection and the value of the clones as parents (GCA), in several sets of data obtained in the different hybrid trials.
The average coefficients of linear correlation between the replicate data, obtained from the accessions that were repeated twice in the collection, were significant for WBW and for NHP, and the correlations were slightly improved when the yield data were corrected using TS as a co-variable or as a block effect, which indicates that the correction reduces the fixed effects of the environment.
In general, highly significant parental and interaction effects were observed in the progeny trials. However, parent-offspring correlations were non-significant for WBW and NHP for relatively uniform parental clones (*e.g.* local *Comun* clones). However, positive parent-offspring correlations were often observed for genetically variable clones, but only when NHP data from the parental clones were corrected by using TS. The same tendency was observed for WBW, again when corrected using TS, though this was less clear than for NHP.
It is concluded that yield data, corrected for vigour, might represent a more stable trait in germplasm collections than yield itself. Furthermore, NHP data of parental clones that are corrected by TS appear to be a more reliable predictor of the number of pods produced by the progenies than uncorrected parental data.

Introduction

The prediction of the general combining ability (GCA), from the performance of accessions *per se* in germplasm collections, and the methodologies of analyses that can improve the efficiency of evaluation, are important points to be investigated in the optimisation of breeding programmes. The objective of this paper is to compare sets of data obtained from cocoa clones in the CEPEC collection with that of the GCA of the same clones tested in a series of hybrid progeny trials.

Material and methods

Germplasm collection

The clones included in this study are accessions of the CEPEC's germplasm collection, normally cultivated in rows of 10 plants. Twenty-one of these accessions were represented by two rows, each of 10 plants. This collection was planted between 1978 and 1985. The characters analysed were the wet bean weight per plant (WBW) and the number of healthy pods per plant (NHP). Five plants per row (accession) were evaluated for six agricultural years (harvests made April to March between 1988 and 1994). This was before the outbreak of Witches' Broom disease at the CEPEC experimental station, therefore no interference with this disease occurred. All fruits reaching the ripening stage were considered. Trunk diameter of the accessions in the collection was measured in 1996. This was used to calculate the trunk cross-sectional area (TS) for all the clones, and TS was used as a co-variable to correct yield data.

Progeny trials

The following progeny trials established in CEPEC were used in this study: one complete diallel with selfings (trial 1) and seven NC II trials (trial 33, 34, 35, 37, 40, 41 and 42). The parental clones considered in the analyses were:

- trial 1: ICS 1, CC 41, CEPEC 1, SIAL 169 and SIC 19;
- trial 33: IMC 67, POUND 1, and SCAVINA 6 x CEPEC(clones 11, 12 and 14);
- trial 34: SIAL 169, 325, 327, 88, SIC 22, 23, 24, (329 x ICS 1) and PA 150;
- trial 35: ICS 1, ICS 6, IMC 67, UF 613, (UF 667 x SIAL 169), SIC 250, 831 and 891;
- trial 37: ICS 6, SPA 17, UF 296 x EEG 65, SIAL 244 and SIC 18; and
- trials 40, 41 and 42: these trials were analysed together as a NC II design for the female parental clones (AB 1, BE 2, 3, 4 and 5, CA 2, MA 12 and 14, RB 40 and 48) and for the male parental clones (ICS 1, 8, SIAL 325 and SIC 19).

The trials were planted between 1975 and 1983 (trial 1 in 1975, trial 33 in 1979, trial 34 in 1981, trial 35 in 1982, 37 in 1982, and trials 40, 41 and 42 in 1983). The data analysed were the annual average NHP and WBW per plot for the trials 1, 40, 41, 42. and the annual averages for NHP and WBW per plant for the trials 33, 34, 35 and 37. The data analysed concern the years 1986 to 1990 for trial 1, 1982 to 1988 for trial 33, 1985 to 1987 for trial 34, 1984 to 1988 for trial 35, 1985 to 1987 for trial 37, and 1989 for trials 40, 41 and 42.

Analyses

The averages for WBW and NHP for the clones in the collection were obtained from the analyses of more than 500 accessions. The model used considered the following sources of variation: year, the cross-sectional area (TS) of the trunk taken 20 centimetres above the soil, and clone. All were taken as fixed effects, with the exception of the TS, that constituted, with each one of their individual values, the blocks of the model. One alternative model using TS as co-variable was tested (PROC GLM – SAS System, SAS Institute 1988).

To obtain the average GCA values of the progenitors, the trials 1, 33, 34, 35 and 37 were analysed with, as sources of variation: block, year (Y), female parent (F), male parent (M), and interactions (YxF, YxM, FxM). Trial 1 was analysed according to the methodology of Griffing (Cruz and Regazzi 1997; Cruz 1997). The trials 40, 41 and 42 were linked through two common treatments (controls), and could therefore be analysed together as a NC II design. Sources of variation were first: trial, blocks within trial, treatment (hybrid progenies), plants inside treatment and the interactions of trial x common treatments and trial x plants inside the common treatments. Then, using the corrected averages of progenies obtained through this

model, a partial diallel analysis was carried out, according to the methodology of Griffing (Cruz and Regazzi 1997; Cruz 1997).

The statistical method used for the analyses is the GLM procedure of the SAS package (SAS Institute Inc.1988).

Results and discussion

Performance of the accessions *per se* (phenotypic clone values)

The germplasm evaluation process was based on non-randomised plants in the field (the accessions are cultivated in rows of 10 plants). Therefore, the differences observed among accessions include environmental differences that relate to their position in the genebank.

The reduction of the position effects in the differentiation of the treatments can be obtained by covariance analyses (Finney 1957; Cochran and Cox 1957; Steel and Torrie 1980). In this study, the size of the plants has been used as co-variable, since it is likely that unfavourable conditions for yield would also be unfavourable for vegetative growth.

The improvement in the precision of the covariance analyses is associated with the degree of correlation between the co-variable and the main variable. In general, this method is used when the co-variable and main variable are relatively independent, in such a way that the analysis just affects the main variable. Otherwise, the adjustment does not just remove a component of the error, but it also affects the treatment effect, causing difficulties in the interpretation of the results (Smith 1957).

In our study, the averages of the coefficients of linear correlation (Pearson) between the co-variable (TS) and WBW and NHP, for trees within clones (environmental correlation inside lines) were 0.40 and 0.40, respectively. The same coefficients of correlation for all individual plant data (genetic correlation + environmental correlation inside lines + environmental correlation between lines) were 0.31 and 0.25, and for the averages of clones (genetic correlation + environmental correlation between lines) were 0.26 and 0.15. These data indicate that the genetic factors should not have inflated the corrections of the WBW and NHP through the use of TS as a co-variable under our conditions.

Increases in accuracy can also be obtained by the formation of blocks related to the values of the co-variable. In this study, although this procedure resulted in corrected averages very similar to the ones obtained with the covariance analyses (coefficients of correlation of Spearman of 0.991 for WBW and 0.992 for NHP), it showed some superiority in the reduction of the differences between the row averages for those accessions that had been planted in replicated rows,

In order to calculate the relative value of these two methods in improving the genotypic value for the yield data, the 21 accessions represented in the collection by more than one row were analysed separately. Two groups were formed, both with all the 21 genotypes, but each one involving just one of the repeated rows. With the correction for the trunk section (TS) through block effects, the adjusted averages of the accessions obtained in each analysis with the 21 accessions produced coefficients of Pearson correlation ranging from 0.73 to 0.92 for WBW, and from 0.69 to 0.92 for NHP, according to the distribution of the rows between the groups. Without the TS correction, these coefficients ranged from 0.63 to 0.85 and from 0.65 to 0.87 for WBW and NHP, respectively. When applying the covariance analysis without block effects for TS, these coefficients ranged from 0.72 to 0.89 and from 0.70 to 0.89.

These results show the usefulness of the block adjustment technique for the reduction of the position effects. This method has therefore been applied hereafter to calculate the association among values *'per se'* of the accessions in the collection, and their general combining ability in progeny trials.

Progeny trials

In general, there were highly significant effects of the female parents (at 0.1% probability), male parents (at 0.01% probability) and interaction effects (also at 0.01% probability) in the progeny trials 1, 33, 34, 35 and 37. Only for the variable NHP in trial 34 was there no significance for the differences among female parents ($p > 0.1$).

For the group of trials 40-41-42, significant effects for trials (0.01%) were observed as well as significant differences between progenies (at 0.01%) for both yield variables. The interaction effect between control progenies and trials was non-significant for NHP, but was significant for WBW (1%). This interaction for NHP indicates that the control progenies behaved differently in each trial and the same might happen for the other progenies. Therefore, for the parent-offspring correlation for this group of progeny trials, only the variable NHP will be considered hereafter.

For NHP the partial diallel analyses showed significant differences in general combining ability for female and male parents (1%)

Correlation between clone values and general combining ability (GCA)

Number of healthy pods (NHP)

For the more uniform group of parents used, *i.e.* the ones composed of selections from local varieties (male parents in the trials 33, 35 and 37, and females in trial 34), no significant parent-offspring correlations were observed when comparing clone values observed in the germplasm collection with the effects of the male or female clones in the progeny trials. Similarly, no significant parent-offspring correlations were observed when comparing individual clone values with parental effects in trials 40, 41 and 42.

However, in the latter trials, for each of the groups BE, MA and RB, more than one parental clone was used. This allowed comparison, among the averages of these three groups, of the performances as clones and as parents, which appeared to be strongly correlated ($r=0.98$, but not significant at 5% for only three data points). When using the average of the three groups, BE, MA and RB as well as the average of each of the eight parents of the same groups, a significant coefficient of linear correlation of 0.88 (at 0.4% probability) was observed for NHP as measured in the collection, corrected by the trunk section (TS), and the average NHP of each of the parents in the progeny trials.

So, the inefficiency in distinguishing individual genotypes inside homogeneous genetic groups in the evaluation of germplasm is an element that hampers the verification of positive parent-offspring correlations. Furthermore, without correction for TS in the collection, the above parent-offspring correlation is reduced to a non-significant value of 0.67 (7% probability). As will be shown hereafter, these two aspects appear to be a general tendency.

This was the case for parental clones in other trials that included more than one origin. For example, for the male parents of the trials 40,41, and 42, an r-value of 0.86 (14% probability) was obtained for NHP, corrected by TS, and 0.51 (49%) with no TS correction. For the female parents of trial 37, these values were 0.99 (8%) with TS correction and 0.98 (13%) with no TS correction, and for the female parents of trial 35 these values were 0.56 (33%) with TS correction and 0.13 with no TS correction. For the male parents of trial 34 (only two) the r-values were 1.0 (with TS correction) and –1.0 with no TS correction. For the parents of trial 1 (complete diallel), the r-values were 0.66 (7%) for TS corrected data and 0.64 (9%) for uncorrected data.

The only group that did not follow this tendency was the one formed by the female parents of trial 33: IMC 67, POUND 7 and SCAVINA 6, for which the correlation among values of NHP was near to 0 (-0,04).

Wet bean weight (WBW)

For comparisons between parents from different genetic groups, some tendency towards positive correlations between parents and offspring for WBW was observed. However, this tendency was not as clear as that observed for NHP. R-values observed for the female parents of trial 37 were 0.96 (7%), with the use of corrected data, and 0.81 (40%) with the non-corrected data. These values were 0.61 (28%) and 0.22 for the female parents of trial 35 for TS corrected and uncorrected data, respectively. No tendency towards correlation was observed for the parents of the trial 1, the female parents of trial 33 and the male parents of trial 34.

Conclusions

Yield data, corrected for vigour, might represent a more stable trait in germplasm collections than yield itself. Corrected NHP data from parental clones coming from different genetic groups appear to be a more reliable predictor of the number of pods produced by the progenies than uncorrected parental data.

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Correlation between Parental Genotypes and the Yields of their Hybrids and the Productivity of Clones Derived from these Hybrids

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Abstract

The relationships between parental genotypes and yield of their hybrid progenies and that of "hybrid clones" derived from these progenies were analysed based on observations of five different clone trials established in Papua New Guinea since 1995. The trials, established as part of the ongoing breeding programmes, were designed with various objectives, and involve a variety of genetic material and experimental details. The volume of data available from the various trials has also not been uniform. Considerable differences between parental genotypes were found for the proportions with which these were represented in the pedigrees of the selected highest yielding clones in all the five trials, suggesting differences in their combining ability for yield.

Yield data for hybrid families were available for three trials. Correlations of varying levels of significance were observed between the yields of the hybrid families and the derived clones, depending on the nature of the materials tested and the quality of the available data. The correlations improved when yield data for both the hybrid families and the derived clones were available for a longer time in replicated and well executed trials. Higher correlation coefficients were obtained when the average combining ability of the original parental genotypes were considered rather than individual hybrid families. The significance of the data presented is discussed. It is concluded that the knowledge about the combining ability of the parental clones is very important for breeders to make wise decisions about the choice of parents in order to obtain the best possible families. However, the data presented are considered only as preliminary. Additional systematic, relevant and sustainable research in properly constructed and well-executed field trials is required.

Introduction

The phenomenon of heterosis or hybrid vigour is a very powerful breeding tool used to develop hybrid varieties in many plant and animal species. It is first and foremost associated with maize. Most of the concepts and terms used such as General and Specific Combining Abilities (GCA and SCA) evolved from the research done in maize. The empirical discovery of good combining ability between a northern flint population and a southern dent population was probably the most significant event in the history of breeding hybrid varieties. This was followed by the development of the two famous inbred lines, B73 and MO17, that showed excellent SCA. About 70% of the maize hybrids grown in the world during the 1970s were the hybrid B73 x MO17 called by different names by different seed companies.

Inter-population heterosis in cocoa was first demonstrated in Ghana by Posnette (1943) and later confirmed in Trinidad (Montserin *et al.* 1957). Since then, crop improvement in cocoa has relied heavily upon the exploitation of heterosis (Warren and Kennedy 1991). Hybrid seeds currently being released to farmers are often derived from crosses between Upper Amazonian Forastero types and locally selected clonal materials. However, commercial planting of hybrid cocoa demonstrates a high degree of genetic diversity and phenotypic variation for yield (Warren and Kennedy 1991; Efron *et al.* 2003)."Clones are the most efficient means of exploiting genetic variation, which is why they are the planting material of choice

whenever they are technically and financially feasible" (Lockwood 2003). This was recognised at the Cocoa and Coconut Institute (CCI, formerly CCRI) in Papua New Guinea (PNG) as of 1994 (Efron *et al.* 2003), and the development of clones became a major breeding strategy. Broadly, the approach used was to select the clones from progenies of Upper Amazonian x Trinitario hybrids. Accordingly, the term hybrid clones was chosen. Initially, advantage was taken of the existing hybrid progeny trials. However, to achieve further progress, there is a need to have a better understanding of the relationship between the parental clones, their hybrids and the derived hybrid clones.

Simmonds (1996) proposed, based on theoretical considerations, to select clones from only the best few families. However, he indicated that there were no good published data to support his recommendation. Several yield trials with clones derived from hybrid progeny trials were established at CCI. Results from these trials were analysed to study the effect of the parental clones used to develop the hybrids on the yield potential of the derived hybrid clones. Whenever possible, the yields of the hybrid clones were compared with the yields of the original hybrids.

Materials and Methods

The following five trials were analysed:

1) Accelerated hybrid clone development programme
2) CFC local clone observation trial
3) Upper Amazonian clones
4) Trinitario clones
5) Hybrid clones from Progeny Trial 101

The trials are briefly described together with the results.

Results

Accelerated hybrid clones development programme

Progenies of 27 hybrids produced in BAL plantation, Malaysia were included in the first trial (Table 1). Unequal numbers of progenies depending on the availability of seeds were initially planted at a very high density of 10,000 seedlings ha^{-1}. Following the development of the first primary fan branches, 2539 clones were established by budding. The clones were field planted in an observation trial of one replication with four trees per replicate at a density of 1667 trees ha^{-1}. Yield was measured for a period of two and half years from the initial production. A total of 174 clones (6.3%) with yield above 2000 kg ha^{-1} were selected for advanced testing in a replicated trial. The same 27 hybrid families were planted also in a separate hybrid progeny trial in five replications with 20 trees per replication at a density of 833 trees ha^{-1}.

Different numbers of clones were tested from the various crosses due to the unequal numbers of seeds that were originally available (Table 1). The proportions of selected higher yielding clones from the different crosses varied from 1.0% for the cross PA 13 x ICS 1 to 12.7% for the cross NA 33 x NA 149 (Table 1). The three female parents (PA 13, SCA 12 and NA 33) had similar proportions of selected clones. However, the average proportions of clones selected from the male parents varied from 2.3% for QH 441 to 9.7% for NA 149.

There was no correlation ($r = 0.04$) between the proportion of clones selected from the different crosses and the yield of these crosses in the hybrid progeny trial (Figure 1).

Table 1. Number of clones tested and the proportion selected for advanced testing from 27 different crosses in an accelerated hybrid clones development scheme using crosses produced at Bal Plantation, Malaysia

Male Parent	Female Parents						Total	
	PA 13		SCA 12		NA 33			
	No. tested	Selected (%)	No. tested	Selected (%)	No. tested	Selected (%)	No. tested	Selected (%)
NA 149	99	11.1	116	5.2	63	12.7	278	9.7
IMC 23	70	7.1	81	11.5	171	7.0	322	8.5
NA 226	67	7.0	144	9.7	51	7.8	262	8.2
PA 300	51	5.9	71	7.0	83	8.4	205	7.1
BR 25	21	9.5	180	6.1	258	5.4	459	7.0
PBC 123	165	7.3	80	6.3	26	3.8	271	5.8
KA2 106	56	5.4	68	5.9	74	2.7	198	4.7
ICS 1	97	1.0	128	3.1	103	4.9	328	3.0
QH 441	68	2.9	71	1.4	77	2.6	216	2.3
Total	694	6.4	939	6.2	906	6.1		

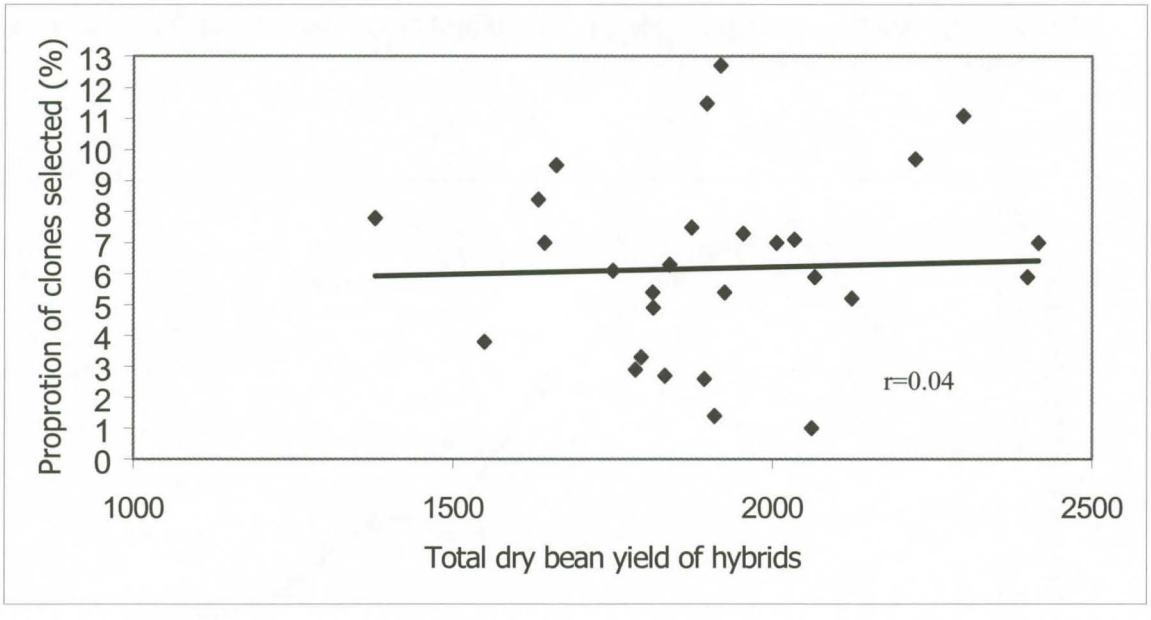


Figure 1. Relationships between the proportion of high yielding clones selected for advanced testing in the accelerated hybrid clone development programme using hybrids produced by BAL Plantation, Malaysia and the average yield of the same crosses tested in a hybrid trial

CFC local clone observation trial

A total of 374 clones were included in the CFC local clone observation trial. The clones were selected from various progeny trials that were established previously at CCI. They were derived from various types of crosses (Table 2). The trial was

planted with one replication of eight trees at a density of 833 trees ha^{-1}. Yield data were obtained for three years (2000 – 2002).

Table 2. Average total dry bean yield during 2000-2002 and the proportions of clones with yields above 4000 kg ha^{-1} in five different types of crosses used in the CFC local clone observation trial

Type of cross	Number of clones	Dry bean yield (kg/ha)	Clones with above 4000 (kg/ha)[1]	
			No.	Proportion (%)
BR Trinitario x Amazonian	128	2805	16	12.5 (3.9)
AK Amazonian x Kew introduction	97	2597	20	20.6 (5.1)
AM Amazonian x Amelonado	57	2235	3	5.3 (0.0)
TK Trinitario x Kew introduction	46	2146	1	2.2 (0.0)
TM Trinitario x Amelonado	31	1325	0	0.0 (0.0)

1) Numbers in brackets represent the proportions of clones with total yield above 5000 kg ha^{-1}

The total yield of the individual clones ranged between 573 kg ha^{-1} to 6988 kg ha^{-1} at approximately normal distribution with a tail of high yielding clones (Figure 2). The clones with yields exceeding 4000 kg ha^{-1} were considered as high yielding. The BR and AK groups had the highest average yields and the highest proportions of selected high yielding clones (Table 2). The effect of the parental clones was further analysed only in these two groups.

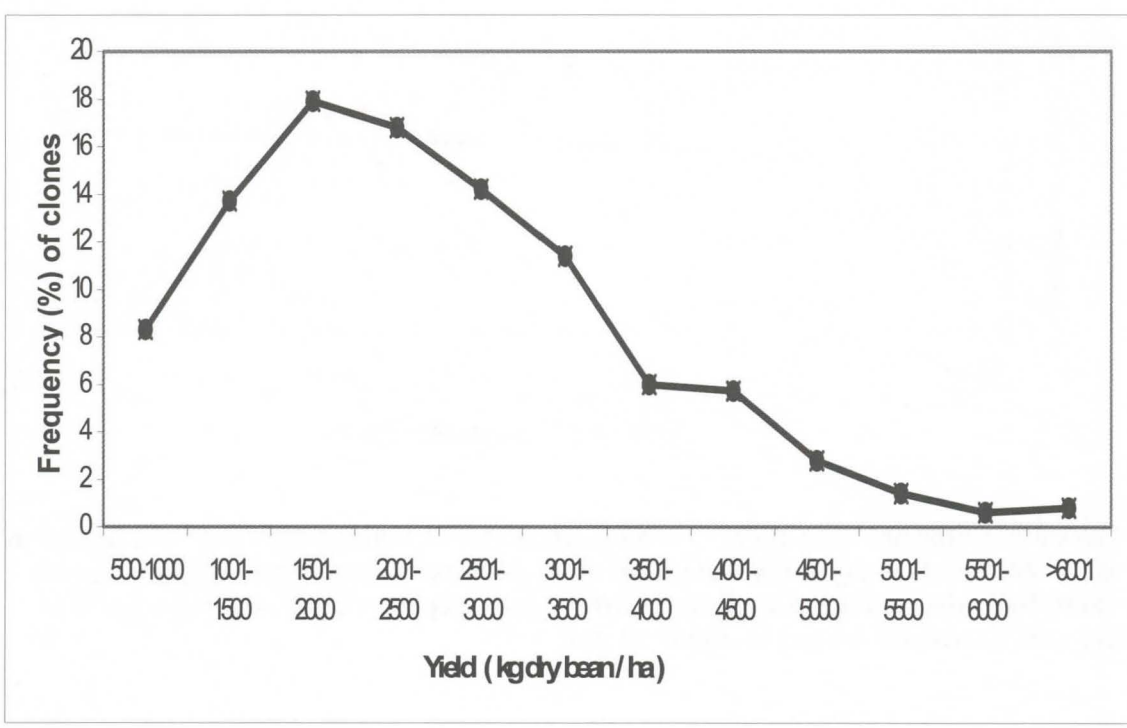


Figure 2. Frequency distribution of total yield obtained by clones in the CFC local clones observation trial during 2000-2002

Four Trinitario clones were used as parents for the BR clones (K82, KA2-106, KA2-101 and K13). More clones were tested with either K82 or KA2-106 as parents (Table 3). However, the average yield and the highest proportion of high yielding clones were obtained when the clone K13 was used as a parent. The other parents in the BR group were unidentifiable individual Amazonian progeny trees.

Similarly, there were four Amazonian clones in the AK group. Most of the clones tested had KEE 42 or KEE 43 as a parent (Table 4). KEE 43 combined particularly well with the introduced clones to produce the highest average yield and the highest proportion of high yielding clones. Only six of the clones tested had KEE 48 as a parent. These clones had the lowest average yield.

Six introduced clones were used as parents in the AK group (Table 5). The production of these clones was tested from 1985 to 1991. Their rank, based on yield during this period, was similar to the rank of the clones derived from them as parents in the AK group. Thus, UF11 and SIAL 93 were the highest yielding, both as clones and as parents, while REDAMEL 1-31 and SPEC 69-9 were the lowest yielding as clones and as parents.

Upper Amazonian clones

The Upper Amazonian clone trial was planted for the purpose of population improvement. The clones were developed from selected progeny trees of a diallel set of crosses between eight selected KEE clones (Table 6). A total of 424 clones were planted in three replications with nine trees/rep at 833 trees ha^{-1}. Yield results were obtained for three years (2000 – 2002).

Table 3. Average total dry bean yield of BR clones developed from Trinitario parents and the proportions of clones with yield above 4000 kg ha^{-1} during 2000-2002

Parental Clone	No. clones	Dry bean yield (kg/ha)	Clones with above 4000 (kg/ha)[1]	
			No.	Proportion (%)
K13	13	3180	3	23.1 (7.7)
KA2-101	10	3050	1	10.0 (0.0)
K82	51	2753	5	9.8 (3.9)
KA2-106	54	2747	6	11.1 (1.9)

1 - Numbers in brackets represent the proportion of clones with total yield above 5000 kg/ha

Table 4. Average total dry bean yield of AK clones developed from Amazonian parents and the proportions of clones with yield above 4000 kg ha^{-1} during 2000-2002

Parental Clone	No. clones	Dry bean yield (kg/ha)	Clones with above 4000 (kg/ha) [1]	
			No.	Proportion (%)
KEE 43	36	3157	14	38.9 (11.1)
KEE 47	11	2714	2	18.2 (0.0)
KEE 42	38	2303	3	7.9 (0.0)
KEE 48	6	1982	1	16.7 (16.7)

1 - Numbers in brackets represent the proportion of clones with total yield above 5000 kg/ha

The average annual yield of the clones derived from the individual crosses ranged between 803 kg ha^{-1} (KEE 47 x KEE 52) to 1303 kg ha^{-1} (KEE 12 x KEE 48). The highest proportion of selected clones with average annual yield above 1400 kg ha^{-1} was obtained for KEE 12 (21.4%) followed by KEE 22 (17.2%) and KEE 48 (16.2%). The lowest proportion was found for the clone KEE 52 (Table 7). Due to a volcanic eruption there was not sufficient information about the performance of the original hybrids.

Table 5. Average total dry bean yield during 1985-1991[1] of introduced clones from Kew Gardens, the average yield of clones derived from them and the proportion of clones with yield above 4000 kg ha^{-1} in the AK group

Parental clone	No. clones	Av. dry bean yield (kg/ha) [2]		Clones with above 4000 kg/ha	
		Clone	Parent	No.	Proportion (%) [3]
UF 11	24	4772	2945	6	25.0 (8.3)
SIAL 93	23	5881	2841	6	26.1 (8.7)
UF 168	7	3832	2780	2	28.6 (0.0)
POUND 5C	18	3862	2779	5	27.8 (5.5)
REDAMEL 1-31	7	1680	2111	0	0.0 (0.0)
SPEC 160-9	10	1584	2013	1	10.0 (0.0)

1 - Source: Cocoa Breeding Annual Report 1991 - 1992
2 - Comparative yields of the clones during 1985 - 1991 and the average yield of clones derived from them during 2000 – 2002
3 - Numbers in brackets represent the proportion of clones with total yield above 5000 kg/ha

Trinitario clones

The Trinitario clones were selected from a Trinitario x Trinitario progeny trial of diallel crosses between 10 local Trinitario clones. Yield data from this trial were available for five years from 1987 to 1991 (unpublished).

Table 6. Pedigrees of KEE Upper Amazonian clones

Clone	Pedigree	Clone	Pedigree
KEE 12	NA 33 x NA 34	**KEE 43**	POUND 7c x POUND 26a
KEE 22	PA 100 x PA 20	**KEE 47**	PA 124 x PA 149
KEE 23	PA 207 x PA 138	**KEE 48**	PA 179 x PA 132
KEE 42	POUND 7 x POUND 21	**KEE 52**	IMC 67 x IMC 46

A total of 216 clones were established, 152 of them were planted in a trial with three replications and 64 clones were planted only in one replication. There were nine trees per replication at a density of 833 trees ha^{-1}. Yield was measured for three years (1999 – 2001). Later, the trial was terminated due to very heavy attack by longicorn (Efron and Epaina 2003). Thirty-one clones with a total yield above 2000 kg ha^{-1} were selected (Table 8).

The 10 parental Trinitario clones differed as donors for the average total yield and the proportion of selected high yielding clones. The clone K23 was a donor for the highest yield and the highest proportion (28.6%) of selected high yielding clones.

It was followed by the clones K20, K6 and 58/24. The lowest donors were KT140, K82, K13 and KA2-101.

A low but significant correlation (r=0.38*) was found between the yield of the original Trinitario x Trinitario hybrids and the average yield of the clones derived from them (Figure 3). The correlation coefficients were higher when the average yield of the parental clones in hybrid combinations was compared with the average yield of the derived clones (Figure 4A) or the proportions of high yielding clones having the same parental clone in their pedigree (Figure 4B).

Hybrid clones from Progeny Trial 101

Progeny trial 101 included 54 Trinitario x Amazonian hybrids planted in four replications with 16 trees per replicate. The so-called "SG2" hybrid mix was released in 1988 based on the results obtained from this trial.

Two hundred and ten individual mother trees were selected and cloned from the trial based on 10 years of yield data. The clones were planted in a replicated trial of three replications with nine trees per replicate. High yielding clones with an average of above 1500 kg $ha^{-1}year^{-1}$, based on four years of yield data were selected as high yielding clones.

Both the Trinitario and the Amazonian parental clones differed as donors for the proportions of high yielding selected hybrid clones (Table 9). The highest proportions of selected clones were obtained with the Trinitario clones K24-102 and KA2-106 and the Amazonian clones KEE 43 and KEE 12 in their pedigrees. KA2-105 and KA2-101 were the lowest donors for yield potential among the Trinitario parental clones whilst KEE 47, KEE 22 and KEE 5 were the poorest donors among the Amazonian clones.

The average yield of the clones was highly significantly correlated (r=0.57**) with the yield of the original hybrids (Figure 5). Higher correlation coefficients were obtained when the average yield of the parental clones was compared in hybrid combinations with the yield of the derived hybrid clones (Figure 6A) or with the proportions of high yielding selected hybrid clones (Figure 6B).

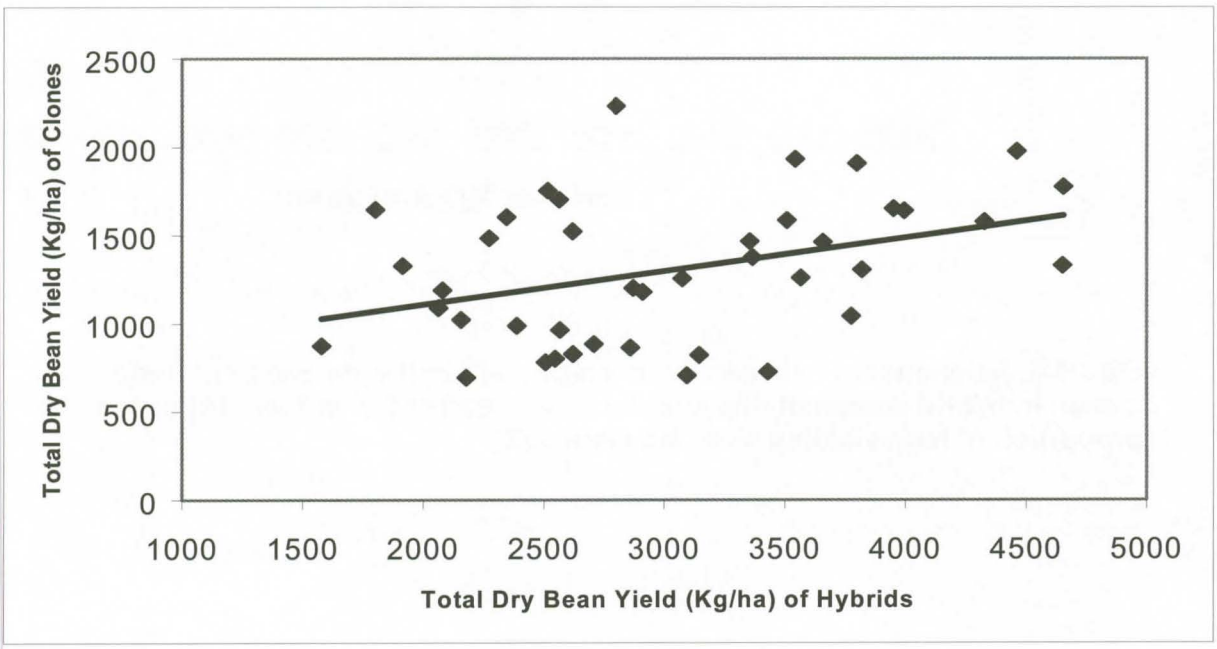
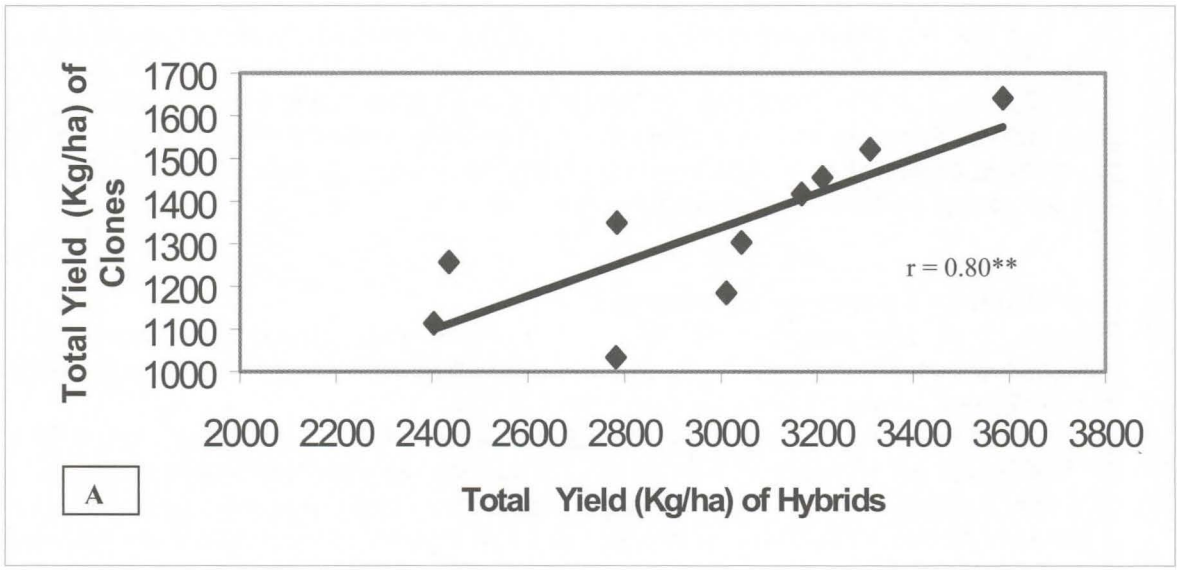


Figure 3. Relationship between the yields of the Trinitario x Trinitario hybrids and the clones derived from them



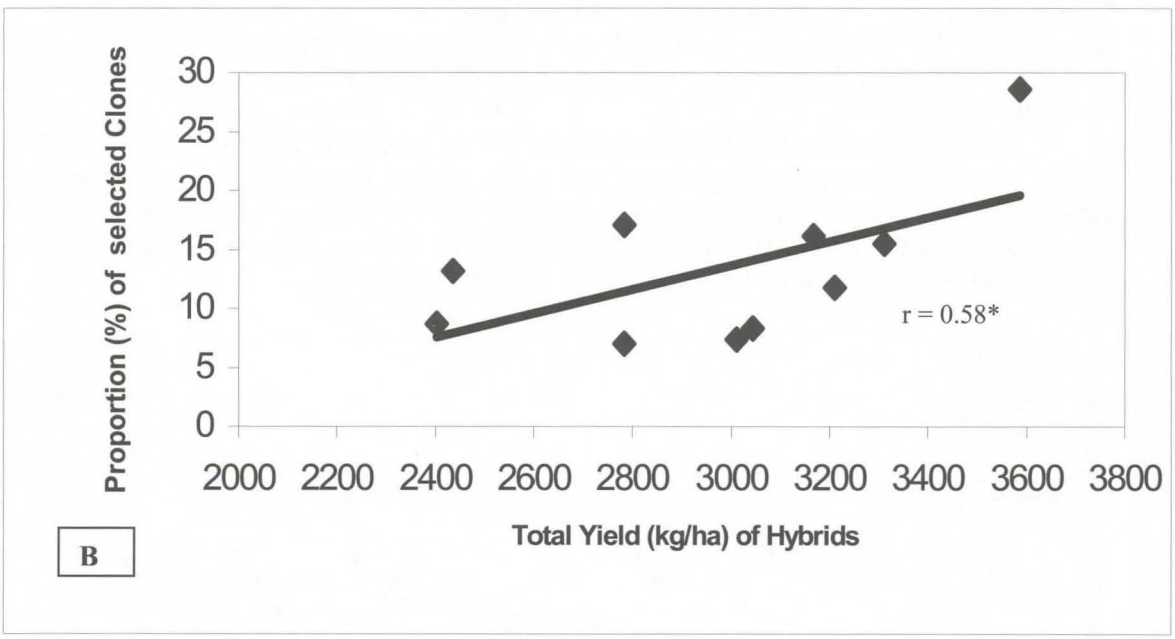


Figure 4. Relationship between the average yield of the Parental Trinitario clones in hybrid combinations and the clones derived from them (A) or the proportion of high yielding selected clones (B)

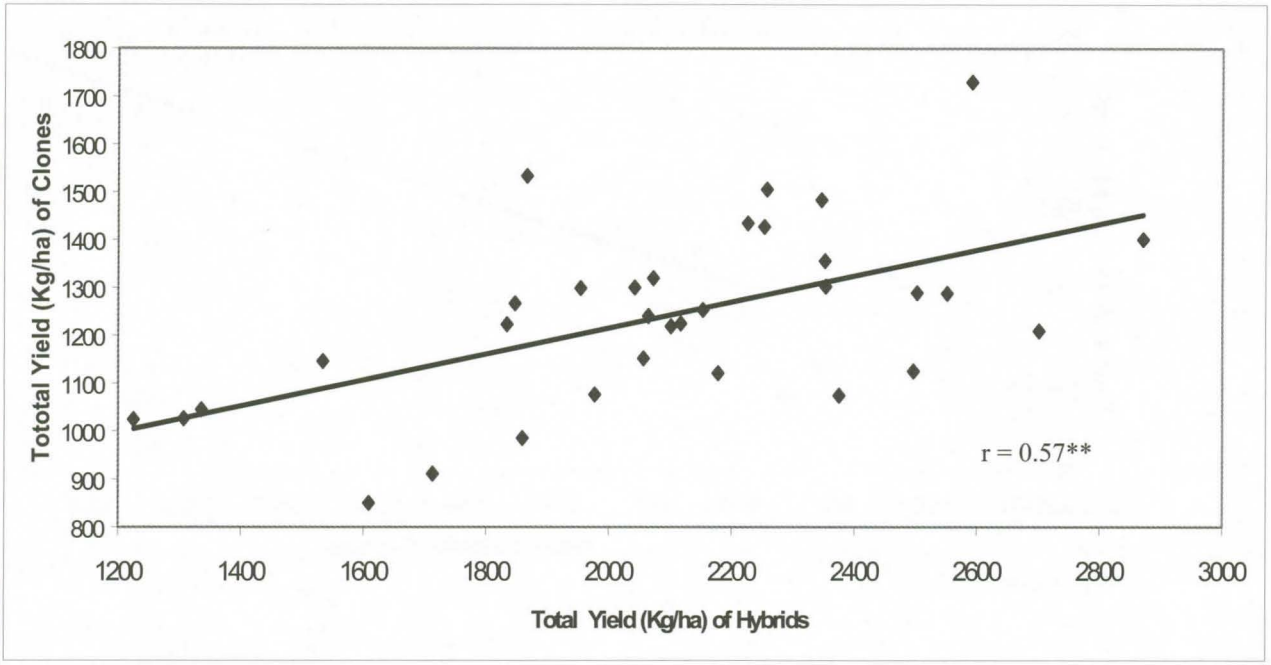
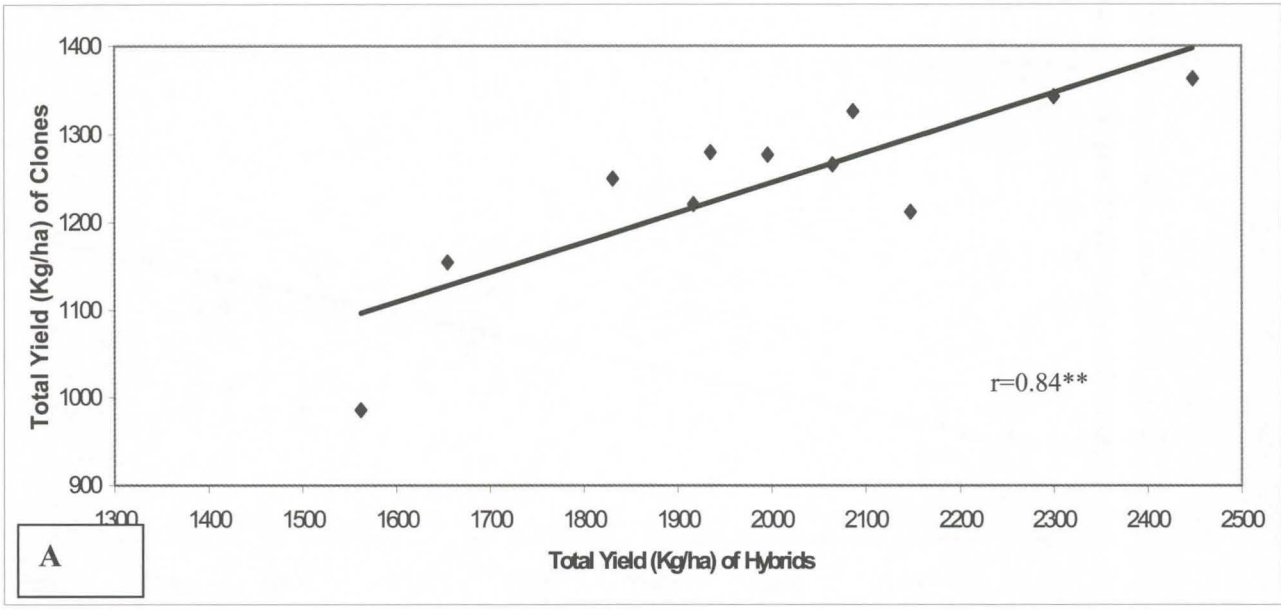


Figure 5. Relationship between the yield of Trinitario x Amazonian hybrids and clones derived from them

Table 7. Average annual yield of clones developed from progenies of diallel crosses between eight Upper Amazonian parents and the proportion of clones with yield above 1400 kg ha^{-1}

KEE no.	KEE number							Average	Proportion (%)
	22	23	42	43	47	48	52		
12	1252 (13)	1174 (13)	1106 (12)	937 (3)	993 (8)	1303 (4)	975 (8)	1106 (61)	21.4
22		1056 (10)	1107 (12)	1066 (7)	1210 (10)	1048 (6)	978 (6)	1102 (64)	17.2
23			945 (10)	874 (8)	897 (4)	1028 (3)	936 (7)	987 (55)	10.9
42				938 (8)	995 (9)	1166 (9)	1023 (4)	1040 (64)	7.8
43					930 (8)	1156 (5)	992 (9)	985 (48)	10.4
47						825 (3)	803 (6)	950 (48)	8.3
48							966 (7)	1070 (37)	16.2
52								953 (47)	2.1

( ) number of clones tested



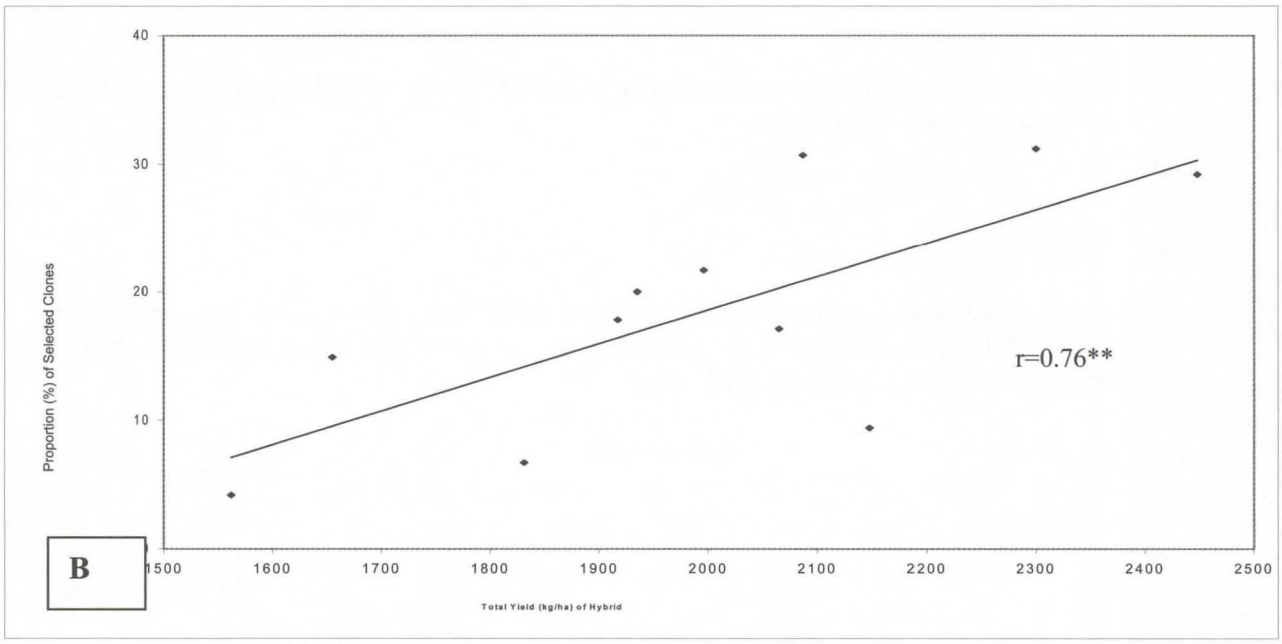


Figure 6. Relationships between the average yield of parental clones in hybrid comparisons and in clones (6A) and proportions of the selected high yielding clones (6B) in Trinitario x Amazonian crosses

Table 8. Average total yield of Trinitario clones derived from diallel crosses between 10 Trinitario parental clones and the proportions of high yielding selected clones

Parental clones	No. clones tested	Av. total dry bean yield (kg/ha)	Selected clones	
			No.	%
K23	56	1641	16	28.6
K20	38	1522	9	15.5
KA5-201	34	1456	4	11.8
K6	37	1417	6	16.2
58/24	35	1350	6	17.1
K13	36	1304	3	8.3
KA2-106	53	1257	7	13.2
K82	54	1186	4	7.4
KA2-101	46	1113	4	8.7
KT 140	43	1034	3	7.0

Table 9. Average annual yield and proportions of clones with yield higher than 1500 kg/ha of the parental clones in a Trinitario x U. Amazonian hybrid clone trial

Parental clone	Type	No. clones tested	Av. Yield (kg/ha)	Proportion selected (%)
K82	Trinitario	56	1221	17.8
KA2-101	Trinitario	32	1212	9.4
KA2-106	Trinitario	48	1343	31.2
KA2-105	Trinitario	15	1250	6.7
K24-102	Trinitario	12	1418	33.3
KT 140	Trinitario	47	1155	14.9
KEE 5	U. Amazonian	12	1353	8.3
KEE 6	U. Amazonian	35	1266	17.1
KEE 12	U. Amazonian	48	1363	29.2
KEE 22	U. Amazonian	25	928	8.0
KEE 23	U. Amazonian	20	1280	20.0
KEE 42	U. Amazonian	23	1277	21.7
KEE 43	U. Amazonian	23	1326	30.7
KEE 47	U. Amazonian	24	986	4.2

Discussion

CCI has modified its breeding strategy to emphasise the development of hybrid-derived clones (hybrid clones) to be released as polyclonal varieties to farmers. The hybrid vigour expressed in crosses between the Upper Amazonian and locally adapted Trinitario germplasm was used to develop the two released SG1 and SG2 hybrids. The same source of crosses was used initially to develop the first set of hybrid clones for testing. It was the fastest possible way since hybrid progeny trials had already been established previously. As a result, it was possible to release two polyclonal varieties of hybrid clones, HC1 – B (with big trees) and HC1 – S (with small trees) in early 2003. These two varieties were considered as first generation varieties of hybrid clones, and further progress can be achieved by a well designed and implemented breeding programme.

The choice of parents to develop both improved hybrids and improved hybrid clones is of fundamental importance. They should have favourable general

combining ability to provide a high frequency of high yielding clones. This was well illustrated by the results presented in this article. In all the five experiments analysed, there were considerable differences in the combining ability of the parental clones that provided different proportions of high yielding hybrid clones. The relationships between the yields of the hybrids and the clones derived from them or the proportions of high yielding clones is of particular interest. In the first experiment of the accelerated hybrid clones development programme, there was no correlation between the yield of the hybrids and the proportion of high yielding clones derived from them. The clones were tested in an unreplicated trial of only four trees per clone at a different density to that used for the hybrids. Yield results for both the hybrids and the clones were available for only two years. However, in the last two experiments of the Trinitario x Trinitario and the Trinitario x Amazonian clones where the clone trials were replicated and yield results were available for a longer time (3 and 4 years, respectively), the performances of the clones were significantly correlated with the yield of the hybrids. Higher and highly significant correlations were obtained when the effects of the parental clones were considered. Thus, the data obtained support Simmond's (1996) family selection concept provided that the families are selected based on well designed trials executed for a sufficient length of time.

Information on the combining ability of parental clones is very important to the breeders. It should assist them in developing improved families for testing. It is particularly important when an accelerated clone development programme is considered (Efron *et al.* 2002). Moreover, cocoa breeding is still largely dependent on clones from the IMC, NA, PA and SCA populations collected by Pound in Peru (Warren and Kennedy 1991). Breeding achievements will be stagnant as long as the same clones are being used repeatedly. Improvement of these materials by various selection schemes is essential for achieving further progress. The information about the combining abilities of the clones would permit the breeders to make informed decisions with regard to which clones should be included in population improvement schemes.

Only yield potential was analysed based on data from several trials in PNG. The same principle can and should be employed for other variables such as disease resistance, vigour and pod characteristics to assess fully the potential value of clones as parents. Most of the parental clones used were locally selected clones of no specific interest to other breeding programmes, but other breeders can follow a similar approach to analyse their trials providing adequate data are available. However, the type of information available to individual programmes can be limited if the trials are not well designed and executed. Systematic, larger scaled, sustainable research in properly constructed and executed field trials is required in order to achieve meaningful progress in cocoa breeding.

Acknowledgement

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The Relationship Between Vigour, Yield and Yield Efficiency of Cocoa Clones Planted at Different Densities

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Abstract

The potential vigour of cocoa trees is under genetic control. Therefore, clones of different tree sizes can be developed by breeding. When polyclonal varieties are being used, it is important that the clones are of similar potential vigour to reduce interplant competition and to optimise agronomic recommendations. In particular, clones of different vigour may have different optimal planting densities. Accordingly, the clones used in the cocoa breeding programme of Papua New Guinea (PNG) are usually divided into big, intermediate and small size groups. An experiment with nine advanced clones, each of the three size groups and two common controls was planted at two locations, Tavilo in East New Britain Province and Hawain in East Sepik Province. Each group was planted as a separate sub-trial at two densities (625 and 1000 trees ha^{-1}) in a split plot design of four replications with 12 trees/replicate. The results showed that the small clones responded better to the increased density compared to the big and intermediate clones. Planting the small clones at the higher density was beneficial economically. Selected small and big clones planted in a second experiment in fertile soil responded similarly to increased density from 625 to 1283 trees ha^{-1}. The difference in the vigour of the three groups was confirmed by measurements of trunk circumference, which was significantly reduced at the higher density. Annual yield efficiency, calculated from the trunk cross-sectional area was the highest for the small clones at both densities. The relationship between yield, vigour and yield efficiency is important in clonal evaluation trials planted at one density. Selection of clones based only on yield potential will tend to favour the more vigorous clones. Examples are provided from the International Clone Trial and the Local Clone Observation Trial of the CFC/ICCO/IPGRI International cocoa project on Cocoa germplasm conservation and utilisation- a global approach.

Introduction

The importance of rootstocks that control tree size was discussed by Purdy and Eskes (2002). The first example of a dwarfing rootstock in cocoa was described by Efron *et al.* (2002). However, cocoa shows considerable genotypic variability in morphological and physiological traits associated with yield (Yapp and Hadley 1994). Thus, since the potential vigour of the tree is under genetic control, cocoa trees of different tree sizes can be developed by breeding.

Harvest index or yield efficiency, defined as the ratio of cumulative yield to cross-sectional trunk area, was used in several studies of tree crops (Hill *et al.* 1987; Larsen *et al.* 1992). Daymond *et al.* (2002) applied a similar concept in cocoa, whereby yield over a discrete period of time was considered in relation to trunk growth over the same period of time.

The Cocoa and Coconut Institute (CCI, formerly CCRI) in Papua New Guinea (PNG) is emphasising the development of hybrid clones to be released as poly-clonal varieties to farmers. It is important that the clones within a variety are of similar potential vigour to reduce interplant competition and to optimise agronomic recommendations. In particular, clones of different vigour may have different optimal

planting densities. Accordingly, the clones used in CCI's cocoa breeding programme are usually divided into big, medium and small size groups.

Two poly-clonal varieties, HC_1-B (big) and HC_1-S (small), were officially released to farmers in early 2003. The clones were selected for release based on multi-locational testing at two planting densities. In addition, in preparation for the release, an agronomy trial was established with a selected number of clones to test the effect of four shade treatments and four planting densities in order to formulate agronomic recommendations to farmers. The following paper presents and discusses the results obtained in the two experiments.

Materials and Methods

Multi-location trial

Twenty-nine Upper Amazonian x Trinitario hybrid clones and two Trinitario clones (K82 and KA2-101) were included. The clones were divided on the basis of visual observation into big, intermediate and small size categories and tested in separate sub-trials at the same sites. The Trinitario clones were used as common controls for the three sub-trials. Each sub-trial was planted at two densities (625 and 1,000 trees ha^{-1}) in a split plot design with densities as main plots and clones as sub-plots. There were four replications of each density for each clone with 12 trees per plot. The trial as described above was planted at two locations:

1) At Tavilo, East New Britain Province, a major cocoa producing area with fertile volcanic soil and moderate rainfall of about 2,500 mm per annum. The trial was planted in a cleared cocoa block under *Gliricidia* shade during 1995.
2) Hawain, East Sepik Province, in the western part of the country with sandy loam soil and about the same amount of rainfall as in Tavilo. The trial was planted in 1996 in a newly cleared forest area under *Gliricidia* shade.

Dry bean yield was estimated by harvesting and counting the pods fortnightly. Pods and wet bean weights were measured several times during periods of peak harvest using all the pods harvested per plot. A uniform rate of 30% was used to convert wet beans to dry beans. The relative vigour of the trees was verified in Tavilo by measuring trunk circumference 20 cm above the ground in 2002, seven years after planting. Yield efficiency was calculated by dividing the dry bean yield by the trunk cross-section (cm^2).

Agronomy trial

The agronomy trial was planted only in Tavilo in an area that was under heavy *Gliricidia sepium* shade for four years. Four shade treatments as main plots, four planting densities as sub-plots and three vigour groups of four clones, each as sub-sub plots were included in a split-split-plot design with four replications per treatment. Dry bean yield and yield efficiency were estimated as in the multi-location trial. Trunk circumference was measured annually. Results only for the released big and small clones are presented as an average of the four shade treatments.

Clone trials

The CFC/ICCO/IPGRI (CFC) International Clone Trial was planted at Tavilo in six replications of eight trees per replication, and the Local Clone Observation Trial was planted at one replication of eight trees. Both trials were planted at one density of 833 trees ha^{-1}.

Results

Multi-location trial

The average yield of all the clones in the three sub-trials was always higher at the density of 1000 trees ha^{-1} than at 625 trees ha^{-1} (Table 1). However, the magnitude of the difference between the high and low densities varied according to the size of the clones. The response to increased density was highest in the small clones with a total increase of 1693 kg ha^{-1} (25.0%) in Tavilo and 2022 kg ha^{-1} (41.1%) in Hawain. It was followed by the intermediate (10.8% and 29.6%, respectively) and the big clones (10.3% and 18.3%, respectively) at the two sites.

The magnitude of the difference between the two densities was age dependent (Figure 1). In the first year of production, the yields at the high density were higher by 60-70% except for the small clones in Tavilo where the difference was 93.7% (Table 1). As the trees became older, the magnitude became smaller with a sharper decrease between year one and two. At Tavilo, the yield of the small clones at high density was always significantly higher than at low density. However, the difference between the two densities was significant only in the first three years for the big clones. At Hawain, the yields at high density were always significantly higher than at the low density (Table 1).

Trunk circumference of the individual clones ranged from 31.5 cm for the clone 23-6/1 (small) to 44.9 cm for 38-8/2 (big). As groups of clones, the average trunk circumference was 41.6, 36.9 and 35.3 cm for the big, intermediate and small clones, respectively (Table 2). High density planting reduced the average trunk circumference by eight percent (approximately 3.0 cm). Usually, the measurements within treatments were very uniform, resulting in a low C.V. of 5.2%.

A comparison between the total yield of the small and big clones showed that at the higher density the yield of the small clones was higher at Tavilo by 923 kg ha^{-1} and in Hawain by 543 kg ha^{-1} (Table 3). However, at the lower density, the yield of the small clones was lower by 62 kg ha^{-1} at Tavilo and by 490 kg ha^{-1} at Hawain. The annual yield efficiency was higher in the small clones than in the big clones. Similarly, it was higher at the high density than at the lower density for both the big and small clones.

Agronomy trial

The average yield of the small clones was always higher than the yield of the big clones (Figure 2). In both groups it was increased from 1998 to 2000. In 2001, the yield was reduced by about 25%, and in 2002 it was increased again to approximately the same level as in 2000. A very similar pattern was obtained at the four different densities. Trunk cross-sectional area was also increased with time (Figure 3). It was always higher in the big clones than in the small clones. The increased planting density reduced the cross-sectional area in both the big and the small clones (Figure 4). Yield efficiency was slightly increased from 1998 to 2000. Later it was reduced, particularly in 2001 (Figure 5).

Yield (Figure 6) and yield efficiency (Figure 7) were increased linearly with the increased density. They were both higher in the small than the big clones, but the response of the two groups to increased density was very similar.

International Clone Trial (CFC/ICCO/IPGRI project)

The total yield for the first two years of production ranged between 150 kg ha^{-1} (P 30) to 2438 kg ha^{-1} (K72-46/51). Trunk circumference ranged from 24.3 (AMAZ 15-15) to 38.7 cm (T 11). Results from several of the higher yielding local and international clones showed that they differed in yield, vigour and yield efficiency (Table 4). Among the local clones, K72-46/51 was the highest yielding clone with intermediate vigour. This clone had the highest yield efficiency. In contrast, T 11 was the most vigorous clone in the trial with half of the yield efficiency of K72-46/51. Similarly,

AMAZ 15-15 had the lowest trunk circumference (24.3 cm) with a relatively low yield. MAN 15-2 produced a 53.5% higher yield than AMAZ 15-15, but its trunk circumference was 51.4% larger than that of AMAZ 15-15. Accordingly, AMAZ 15-15 had a higher yield efficiency (11.5 kgcm^2) than MAN 15-2 (7.7 kg.cm^2).

Table 1. The effect of planting density on the average yield of small, intermediate and big clones at Tavilo and Hawain during 1997 to 2001

Location	Clone size		Average dry beans yield (kg/ha)						
		Density	1997	1998	1999	2000	2001	Total	D
		High	707*	1480*	2942*	2053*	1286*	8468	1693
	Small	Low	365	1061	2573	1711	1065	6775	
		H:L (%)	193.7	139.5	114.3	120.0	120.7	125.0	
		High	643*	1548*	2486	1871	1108*	7656	748
Tavilo	Intermediate	Low	402	1404	2367	1735	1000	6908	
		H:L (%)	160.0	110.3	105.2	107.8	110.8	110.8	
		High	323*	1267*	2663*	2163	1129	7545	708
	Big	Low	202	1040	2377	2125	1093	6837	
		H:L (%)	160.0	121.8	112.0	101.8	103.3	110.3	
		High		925*	2301*	1721*	1999*	6946	2022
	Small	Low		538	1777	1251	1356	4922	
		H:L (%)		171.9	129.5	137.6	147.4	141.1	
		High		645*	2158*	1556*	1911*	6270	1431
Hawain	Intermediate	Low		395	1597	1308	1539	4839	
		H:L (%)		163.3	135.1	119.0	124.2	129.6	
		High		274*	1590*	2320*	2219*	6403	991
	Big	Low		166	1305	1908	2033	5412	
		H:L (%)		165.1	121.8	121.6	109.1	118.3	

High density, 1000 trees ha^{-1} Low density, 625 trees ha^{-1}
H:L (%) = Relative yield at high density compared with low density (100%)
D = The difference (kg/ha) between the total yield at high density and low density
* = Significantly (5%) higher yield at high density than low density.

Table 2. Average trunk circumference of small, intermediate and big clones at two densities at Tavilo, seven years after planting

Clone size	Trunk circumference (cm)		
	Low density	High density	Average
Big	43.5 A (A)	39.6 A (B)	41.6 A
Intermediate	38.5 B (A)	35.3 B (B)	36.9 B
Small	36.4 C (A)	34.3 B (B)	35.3 C
Average	39.5 (A)	36.9 (B)	

Numbers showing the same letters are not statistically significant at the 5% level (Newman-Keul's test). The letters in brackets refer to the density effect within the size groups

CFC Local Clone Observation Trial

Seventeen clones were identified in the CFC Local Clone Observation Trial with an average annual yield higher than 16-2/3 and 73-14/1, the two highest yielding control clones (Table 5). Nine of the 17 highest yielding clones were classified based on their trunk's cross sectional area as big, six as intermediate and only two as small. Since the trial was only an observation trial with one replication, 19 clones from each size group were selected for advanced testing in a replicated trial. The average yield and the trunk cross-sectional area of the 19 big clones were the highest, but the yield efficiency was the lowest (Table 6). The small clones had the lowest yield and cross sectional area, but the highest yield efficiency.

Table 3. Total dry bean yield and annual yield efficiency[1)] of small and big clones at high and low density at Tavilo and Hawain

	Total dry bean yield (kg/ha)					
Density	**Tavilo**			**Hawain**		
	Small	**Big**	**D**	**Small**	**Big**	**D**
High	8468 (18.6)	7545 (12.1)	923	6946	6403	543
Low	6775 (12.8)	6837 (9.1)	-62	4922	5412	-490

1) Annual yield efficiency was measured only at Tavilo and is given in brackets
High density 1000 trees ha^{-1}; Low density 625 trees ha^{-1}
D – The difference between small and big clones

Table 4. Total yield, trunk circumference and yield efficiency of several clones tested in the International Clone Trial (CFC project)

Clone	Source	Dry bean (kg/ha)	Trunk circumference (cm)	Yield efficiency (kg/cm^2)
K72-46/51	L	2438	29.1	18.0
NAB-11	L	2344	32.1	14.3
T-11	L	2148	38.7	9.0
K78-3	L	2026	28.2	14.6
PA 150 (s)	I	2103	30.2	14.5
P 7	I	1812	33.6	10.1
MAN 15-2	I	1658	36.8	7.7
T85/599	I	1500	27.5	12.4
AMAZ 15-15	I	1080	24.3	11.5

L = Local I = International

Discussion

Improved harvest index or yield efficiency is a major tool used by plant breeders to increase productivity. It is associated with reduced plant size and increased planting density. The 'Green Revolution' in rice and wheat was achieved mainly due to the development of semi-dwarf varieties that were more resistant to lodging. It enabled plant density to be increased, which together with he fertilizer application resulted in dramatic yield increases.

Cocoa shows considerable genotypic variability in morphological and physiological traits associated with yield, including vigour (Yapp and Hadley 1994). Accordingly, hybrids or clones with different potential vigour can be developed. Without competition, bigger trees tend to produce higher yields than smaller trees, but their yield efficiency, measured as yield in relation to vigour, may be lower. Therefore, the optimal planting density can be related to the relative vigour of the trees. Theoretically, assuming no competition between trees, the expected yield at the higher density in the multi-locational trials was 160% (1000:625 x 100) compared with that at the low density. These expected ratios were obtained during the first year of production in the three size

Table 5. Average annual yield, trunk cross-sectional area and yield efficiency of 17 selected highest yielding clones in the International Clone Trial (CFC project)

Clone	Dry bean (kg/ha)	TCSA (cm^2)	Annual yield efficiency (kg/cm^2)	Vigour group
BR36-2-4	2329	164.7	14.1	B
BR413-3-9	2234	161.9	13.8	B
BR43-2-16	2210	116.4	19.0	I
AK56-1-14	1981	148.6	13.3	B
AK57-1-9	1967	123.8	15.8	I
BR111-3-16	1756	172.4	10.2	B
AK54-2-6	1733	105.3	16.5	S
AK76-2-16	1725	103.8	16.6	S
BR16-3-2	1699	120.7	14.1	I
AK54-1-2	1691	120.1	14.1	I
AK56-1-12	1641	166.3	9.9	B
BR23-1-7	1639	132.5	12.4	B
AK54-1-9	1616	126.0	12.8	B
AK54-1-8	1605	136.9	11.7	B
AK62-14	1600	110.4	14.5	I
TK37-2-8	1599	111.0	14.4	I
BR33-3-9	1595	204.7	7.8	B
16-2/3 (c)	1572	118.0	13.3	I
73-14/1 (c)	1569	126.0	12.5	I
37-13/1 (c)	1352	131.2	10.3	B

B = Big, I = Intermediate, S = Small, (c) = Control TCSA = Trunk Cross Sectional Area

Table 6. Relationships between vigour estimated by trunk cross sectional area, yield and yield efficiency of 19 selected big, intermediate and small high yielding clones

Vigour Group	TCSA (cm^2)	Yield kg/ha	YE (kg/cm^2)
Big	152.1	4854	10.8
Intermediate	118.0	4575	12.9
Small	91.4	3986	14.6

TCSA = Trunk Cross Sectional Area YE = Yield Efficiency

groups at both sites, except for the small clones at Tavilo where, for unknown reasons, the difference between the two densities was higher. At this age, the trees were relatively small, their canopy was not closed and therefore, they did not compete with each other. However, by the second year of production the canopy of the trees had closed and they were exposed to interplant competition. As a result, the yield advantage at the high planting density was reduced sharply in year 2 and at a milder rate thereafter (Figure 1). The effect of the interplant competition was related to the vigour of the trees, being smaller in the small clones and larger in the more vigorous trees. At Tavilo, the yields of the big clones in the 4th and 5th years were already similar at both densities.

The differential response of the big and small clones to increased density with time was not observed in the agronomy trial. This trial was planted in a very fertile soil which had a high amount of organic matter and nitrogen since it had been planted with only Gliricidia in the previous four years.

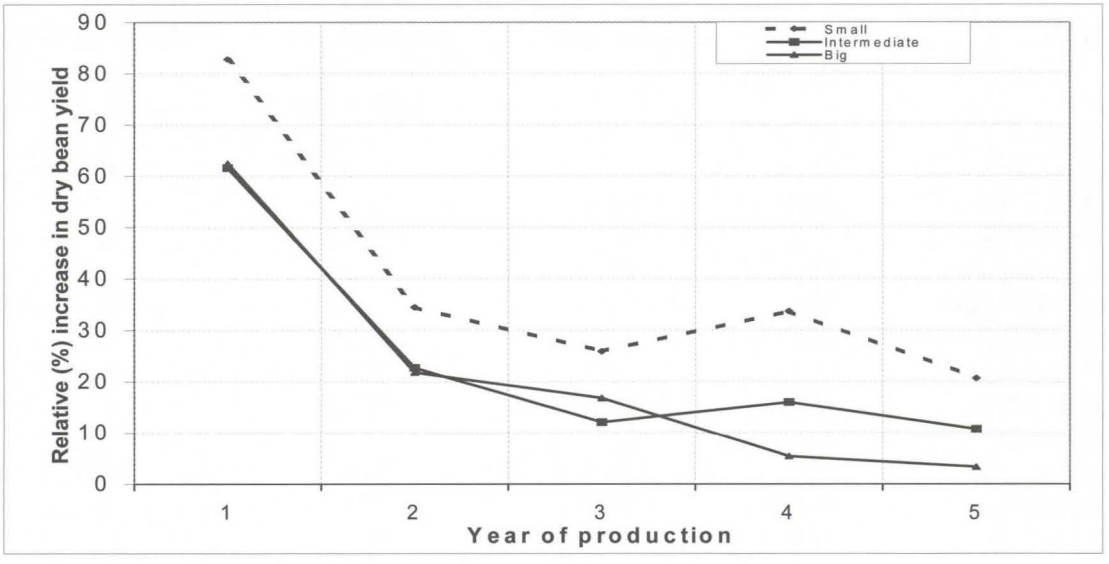

Figure 1. The average effect of tree age on the relative yield increase (%) at high density compared with low density of small, intermediate and big clones in Tavilo, ENB and Hawain, East Sepik

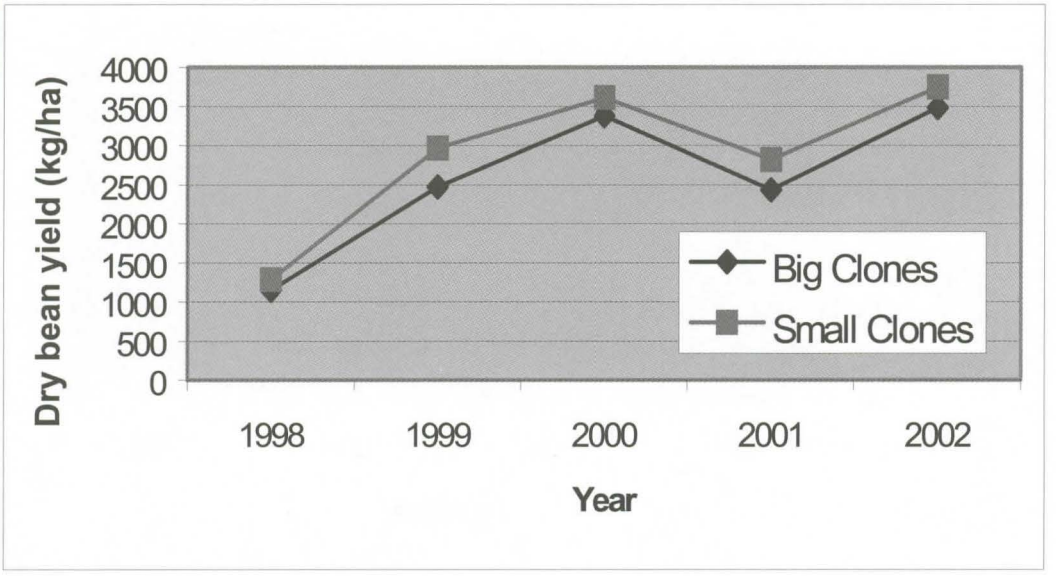

Figure 2. Average yield of big and small clones during 1998 to 2002, Agronomy Trial

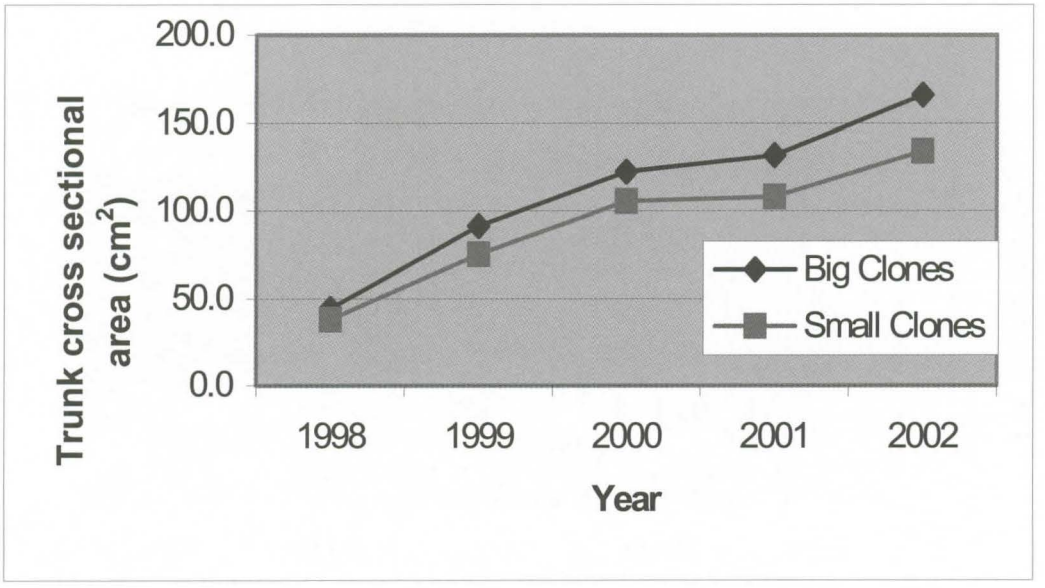

Figure 3. Trunk's cross sectional area of big and small clones during 1998 to 2002, Agronomy Trial

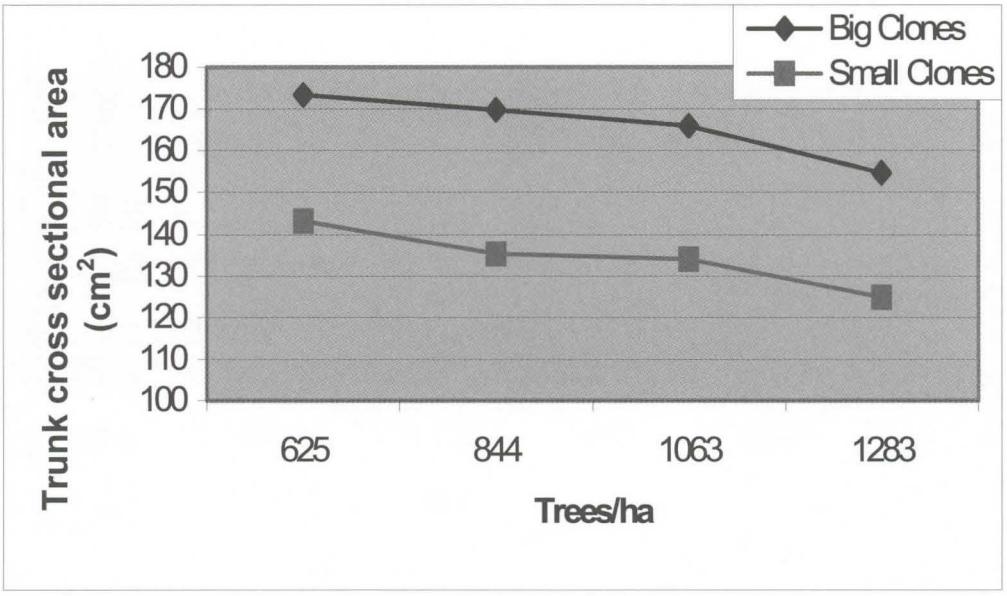

Figure 4. The effect of planting density on trunk cross sectional area in 2002, Agronomy Trial.

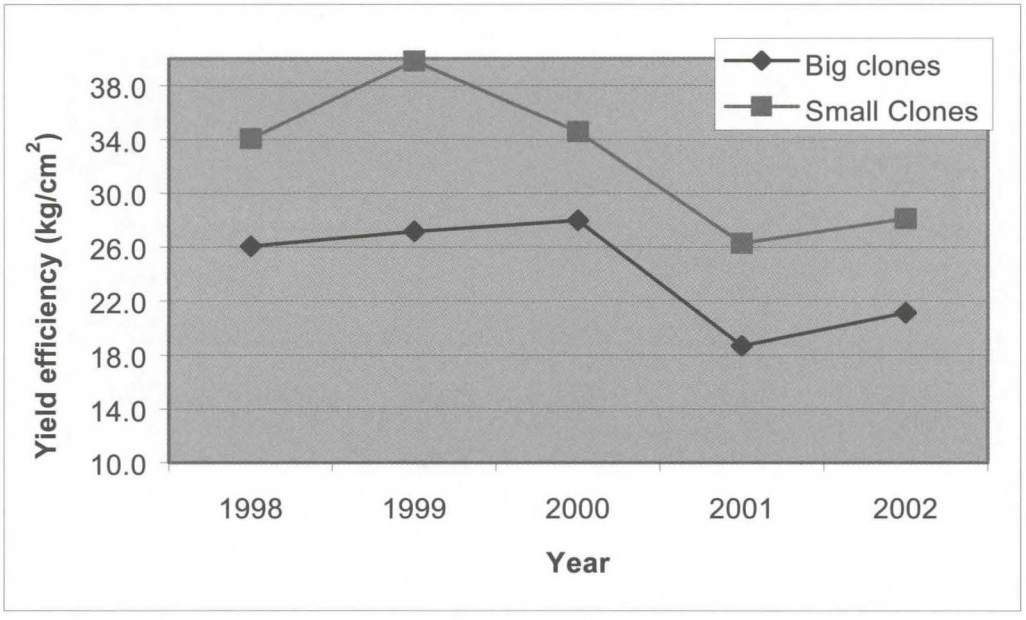

Figure 5. Yield efficiencies of small and big clones during 1998 to 2002, Agronomy Trial

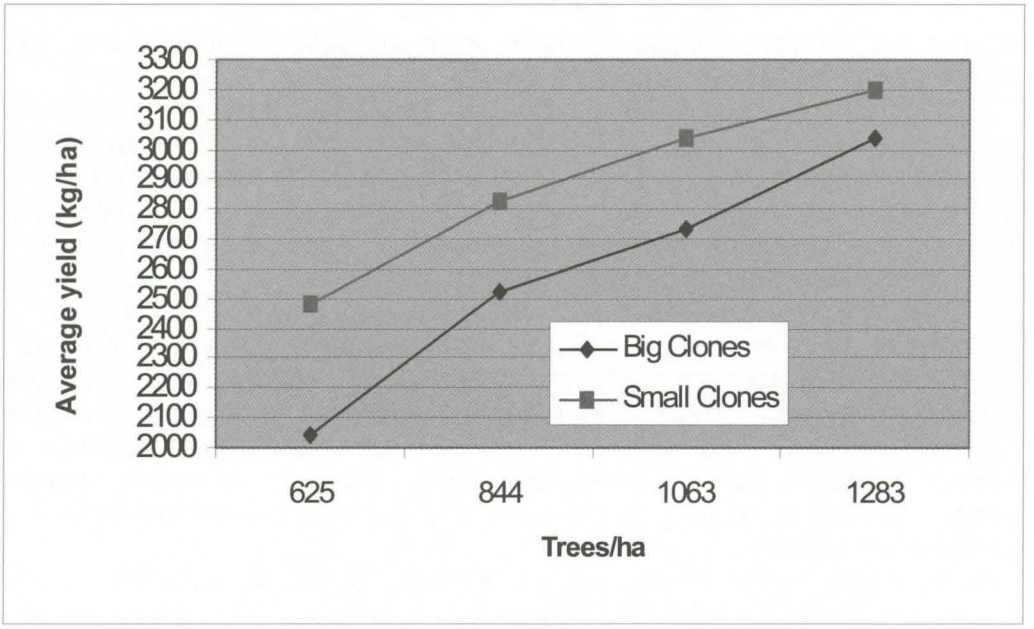

Figure 6. The effect of planting density on the average yield of small and big clones, Agronomy Trial

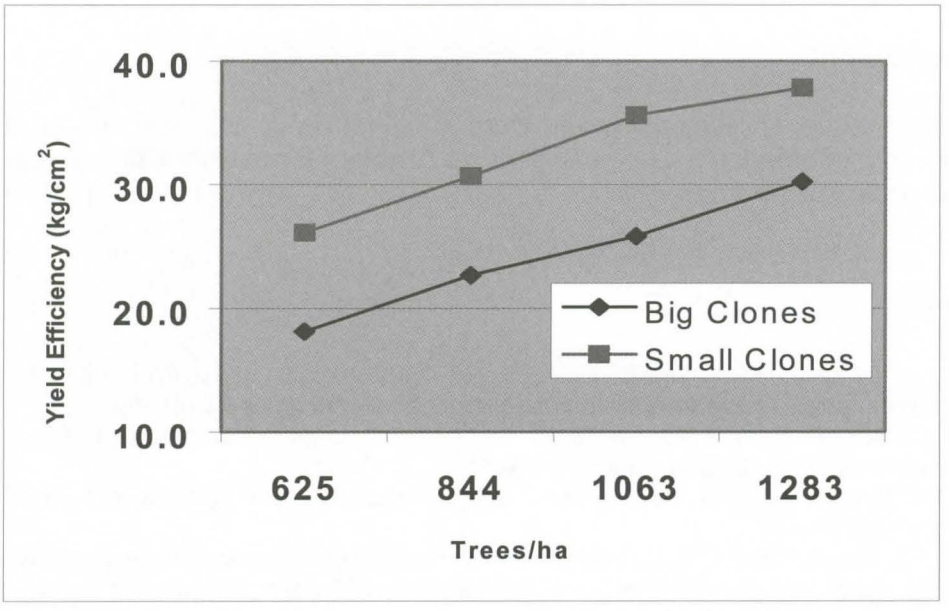

Figure 7. The effect of planting density on yield efficiency of small and big clones

The higher yields obtained in the trial reflect this high fertility. This that optimal density is relative, and probably related to growing conditions such as soil fertility and management level.

The vigour of the clones tested was originally determined by visual observation. The differences in vigour were confirmed during the trials by measurements of trunk circumference. Increased planting density produced higher yields, but at the same time reduced the trunk circumference. As a result, the yield efficiencies were even more responsive to the increased planting density.

Yield efficiency was related to vigour. The small clones had higher yield efficiencies than the big clones. This should be taken into consideration in clonal evaluation trials, since, if only yield potential is considered, the tendency would be to select more vigourous clones as was demonstrated in the CFC trials. Once clones have been selected based on their yield efficiency, they should be planted based on their vigour at an estimated optimal planting density.

A linear increase in yield was obtained in the agronomy trial for the small and the big clones at the range between 625 to 1283 trees ha^{-1}. Possibly, even higher yields could have been realised at a density higher than 1283 trees ha^{-1}. However, the high soil fertility and the good management employed should be considered. Under normal plantation conditions and more so in small-scale farmers fields, the response to increased density could have been different. Moreover, practical management aspects of mobility in the block and higher requirements for pruning should be also considered.

Planting at a higher density was economically beneficial, particularly for the small clones. At Tavilo, the total yield difference was 1693 kg ha^{-1}. This is equivalent to an average of US$338.60 per year at US$1000 per tonne. High density planting incurs additional costs for establishment and management. This includes the cost of additional planting materials, transport, planting, pruning and harvesting. In total, the average annual additional cost was estimated to be US$48.60, leaving an average annual net profit of US$290 ha^{-1}.

Acknowledgement

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Physiological Characterisation of Cocoa Germplasm

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Abstract

Physiological characterisation of cocoa germplasm may be used to select for factors that, under appropriate planting conditions, will enhance yield. Key physiological components of yield are canopy architecture (leaf area index, radiation interception and light attenuation), leaf photosynthesis and partitioning to the yield component (yield efficiency). Methodologies for measuring such parameters range from the use of specialised equipment to more low-tech approaches. Cocoa exhibits considerable genetic variation in a range of physiological and morphological characteristics and there is limited evidence of an interaction between particular genotypes and their environment. This area is one that requires further study and replicated clonal trials in different geographical locations provide one means of achieving this. A better understanding of the physiology of cocoa germplasm will enable more appropriate matching of germplasm to its local environment (in terms of shade, temperature and rainfall) and to husbandry regimes, such as planting density and pruning.

Introduction

Physiological characterisation of cocoa germplasm is relevant to breeders in that it allows for selection of traits that, under particular growing conditions, may increase yield. In this review, we consider the concepts involved in the physiology of yield and the practical means of assessing physiological traits. The extent to which genetic variation in traits underlying yield has been reported in the literature is also considered alongside ways in which breeders can exploit this variation.

Physiological concepts and methodologies

Yield may be considered to be essentially the product of assimilate formation and assimilate partitioning to the reproductive component of the tree. Assimilate production is dependent on the inherent photosynthetic efficiency of the leaves (photosynthetic capacity) and the quantity of solar radiation intercepted by the canopy (Figure 1).

The quantity of solar radiation intercepted by the canopy is a function of the leaf area index (leaf area per unit ground area) and canopy architecture. Canopy Architecture refers to individual leaf size, leaf angle and arrangement. The proportion of radiation intercepted (I) by the canopy can be quantified by equations 1 and 2.

$$I_L = I_o e^{-kL} \qquad \text{(equation 1)}$$

$$I = (I_o - I_L)/I_o \qquad \text{(equation 2)}$$

where I_o and I_L are the radiation flux above and below the canopy respectively, L is the leaf area index of the canopy and k is the extinction coefficient.

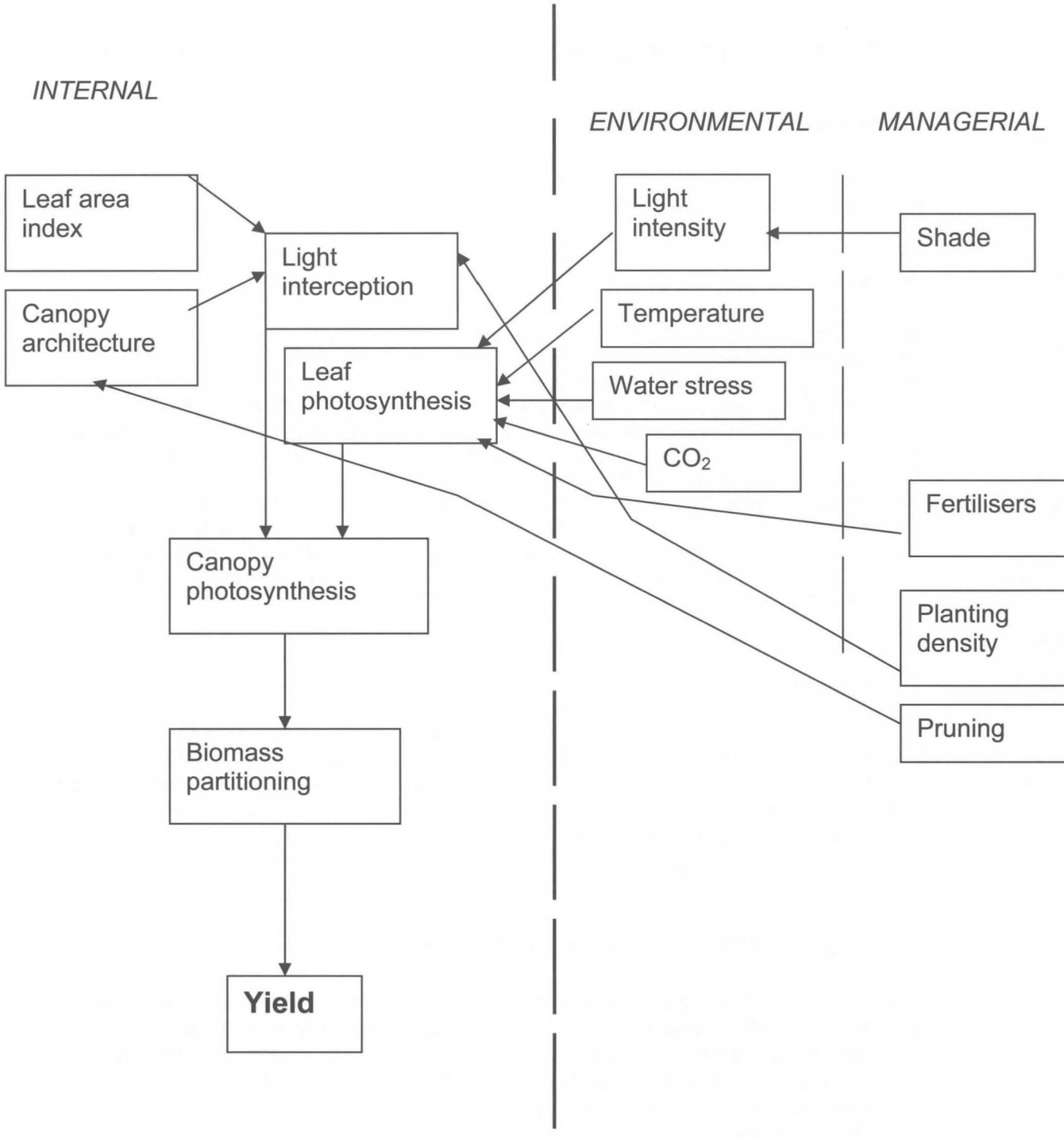

Figure 1. A schematic representation of yield in cocoa

This shows that light is attenuated approximately exponentially through the canopy (measured as leaf area index) but this is also modulated by canopy architecture (measured by light extinction coefficient, k) (Monsi and Saeki 1953).

Various methodologies exist by which canopy characteristics may be assessed. Purpose-built canopy analysers can measure a range of canopy characteristics such as light interception, leaf area index and extinction coefficient. These analysers, however, can be relatively expensive. An alternative, more

economical approach is to use digital photography combined with dedicated computer software to analyse the images produced. Such an approach needs to be standardised in terms of measuring conditions but, nevertheless, provides a more cost-effective alternative to purpose-built canopy analysers.

In the absence of specialised equipment, visual assessments may be used to produce a crude assessment of the canopy. For example, the amount of the light passing through the canopy can be assessed using an index such as that set out in Table 1.

Table 1. Proposed visual assessment scale for light transmission to the ground under cocoa tree canopies (Eskes *et al.* 2000)

Assessment scale	Light transmission
0	0% (no sunflecks)
1	1-5%
2	5-10%
3	10-20%
4	20-40%
5	>40%

Leaf photosynthesis

Photosynthesis can be considered to be the driving force behind plant growth. The most direct means of measuring photosynthesis is to measure carbon dioxide exchange through the leaf using equipment that incorporates an infra-red gas analyser. Essentially, this method measures the rate at which CO_2 is absorbed and assimilated by the leaf and, as such, is expressed in terms of µmol CO_2 m^{-2} s^{-1}. A range of models of photosynthesis equipment are available (all at a high price), some of which are more suited to laboratory situations, whilst others are fully portable. Since their introduction, such equipment has become more portable but also more sophisticated; a number of models have the ability to control light intensity, carbon dioxide concentration, humidity and temperature conditions in the leaf cuvette whilst measuring photosynthesis.

Cocoa is particularly susceptible to environmental conditions such as temperature and water stress (Raja Harun & Hardwick 1988a, 1988b; Balasimha *et al.* 1991), therefore an assessment of genetic variation in photosynthesis needs to involve a standardisation of measurement procedures and conditions under which these are made. Specifically, the plant material should have been grown under the same nutrient regime and should not be subjected to water or vapour pressure deficit stress. Measurements of photosynthetic capacity are often carried out in light-saturated conditions (photosynthetic capacity). Alternatively, a fuller picture may be obtained by analysing the light-response-curves (the photosynthetic response to different light intensities, from darkness to light-saturating).

Chlorophyll fluorescence techniques measure the function of photosystem II (PS II) in the thylakoid membrane (Maxwell & Johnson 2000; Smillie & Hetherington 1983). Rather than directly measuring photosynthesis, the technique gives a measure of the efficiency of photosynthesis. Such a methodology is particularly useful in assessing plant stress responses. This technique has the advantage of being particularly reliable and straightforward making it easy to apply in both greenhouse and field situations.

Biomass Partitioning

The partitioning of assimilates in annual crops is usually measured in terms of harvest index, *i.e.* the ratio of the weight of the yield component to the total weight. In perennial tree crops, a more appropriate concept is that of yield efficiency - the ratio of yield to vegetative growth over a finite period of time. Since destructive measurements are not usually practical for a tree crop, vegetative growth must be estimated by non-destructive means. For temperate crops, such as apple, trunk cross-sectional area (inferred from trunk circumference) is often used (Cannell 1985), although it may be a somewhat crude measure of growth. Yield efficiency, as defined by the ratio of yield to trunk cross-sectional area increase, has proved a useful concept to tree crop breeders (Larsen and Fritts 1982, 1987; Larsen *et al.* 1992).

Genetic variation in physiological traits

A number of studies have demonstrated a high degree of genetic variation in physiological traits amongst cocoa germplasm. The first attempt to characterise a cocoa canopy was made by Alvim (1977). Fractional light interception of a mature hybrid cocoa stand in Bahia, Brazil varied from 0.90 to 0.97 and the mean extinction coefficient was 0.62. From these values, leaf area index values in the range of 3.7 to 5.7 were calculated. Yapp and Hadley (1994) demonstrated considerable variability in canopy characteristics across a range of clones grown at high density at the former BAL plantations in Sabah, Malaysia. Similarly, Daymond *et al.* (2002a) demonstrated a high degree of variability in canopy characteristics between a different set of clones grown under more typical conditions in Bahia, Brazil. The latter study also showed how season can affect the characteristics of cocoa canopies, and highlights the need to repeat such measurements over a period of time to get a more complete picture (Table 2).

Table 2. Comparison of studies of canopy characteristics

Material	Proportion of radiation intercepted (I)	Leaf area index (L)	Light extinction coefficient (k)	Reference
Hybrid	0.90-0.97	3.5-5.7	0.62	Alvim (1977)
Clonal (7 clones)	0.72-0.94	2.3-3.4	0.61-0.95	Yapp & Hadley (1994)
Clonal (10 clones)	0.75-0.88	2.2-3.5	0.63-0.82	Daymond *et al.* (2002)

Studies of photosynthesis in cocoa typically show a photosynthetic rate in the range of 2-5 µmol m^{-2} s^{-1} (Table 3), although higher rates have been found under particularly fertile conditions (Yapp, 1992). Studies of a range of clones grown under similar conditions indicate that some genetic variability in photosynthetic potential exists (Yapp & Hadley 1994; Galyuon *et al.* 1996; Table 3). Whilst higher photosynthetic rates may be correlated with vegetative growth (Yapp, 1992), it should not be assumed that a higher photosynthetic capacity leads to higher productivity since other factors (such as the size of the canopy and the distribution of assimilates) can mask any direct assimilation effect (Daymond *et al.* 2001).

Table 3. Comparison of studies of photosynthesis in cocoa

Internal factor	External factor	Photosynthetic rate (μmol m^{-2} s^{-1})	Reference
Canopy position		2.7 (sun leaves)	Murray, 1940
		1.8 (shade leaves)	
Leaf age		3.8	Lemée, 1955
Tree vigour		2.5-4.4	Hutcheon, 1977
	light	3.9	Raja Harun & Hardwick, 1988a
	temperature, water stress	0.8-1.8	Raja Harun & Hardwick, 1988b
Varietal	light, temperature, water stress	2.0-3.9	Balasimha, 1991
Leaf age	irradiance	2.0-6.5	Balasimha., 1992
Varietal		6.4-8.4	Yapp & Hadley, 1994
Varietal		2.6-3.7	Galyuon *et al.*, 1996

Genetic variability in partitioning to the yield component of cocoa has been demonstrated (Daymond *et al.* 2002b). In this study, a ten-fold difference was found in yield efficiency (*i.e.* the ratio of the yield to trunk cross-sectional growth over a period of 18 months) amongst seven clones and five hybrids. Furthermore, this trait (and thus partitioning) was a relatively stable factor across replicated blocks.

Implications for breeders

Selection of more efficient genotypes

Selection for a high harvest index has been used as a basis for yield improvement in a number of annual crops (Evans 1975). Similarly, yields of temperate tree crops have often been increased through greater partitioning to the yield component (*e.g.* Fallah *et al.* 1994). The considerable variability that exists in cocoa in terms of biomass partitioning clearly offers an opportunity for the breeder.

Appropriate matching of material with husbandry conditions

Breeders can take advantage of the variation that exists in canopy architecture in two ways. Firstly, through the selection of material with low rates of light attenuation through the canopy (low extinction coefficient), *i.e.* for a more open canopy where a large proportion of the canopy is illuminated. Secondly, through the exploitation of variability in canopy architecture and tree vigour to allow more appropriate matching of germplasm with planting density. Yapp and Hadley (1994) demonstrated that in a high density clonal trial (3333 trees ha^{-1}) yield was inversely correlated with extinction coefficient. The implication here is that the reduced light competition of more open canopies allows such material to perform well when planted at higher densities. Including measurements of canopy architecture in established trials designed to investigate the interaction between genotype and planting density (*e.g.* Osei-Bonsu *et al.* 1999) could evaluate the role of light competition in optimising planting density further. Interplant competition (particularly in terms of competition for nutrients and water) will also be affected by tree vigour (Lachenaud and Montagnon 2002). In this respect, an assessment of the efficiency of genotypes could also be used to match more appropriately planting material with density. Additionally, more efficient and thus compact genotypes are likely to require less maintenance pruning.

Appropriate matching of material with the ambient environment

The potential for better matching of genetic material to planting sites based on their response to thermal conditions was demonstrated by Daymond and Hadley (2003), who looked at the early stages of growth in a limited range of material. Growth analysis and chlorophyll fluorescence data indicated some genetic variation in seasonal responses to temperature under controlled greenhouse conditions. Geographically replicated experiments (*e.g.* Eskes *et al.* 2000) have the potential to enhance our knowledge in this area.

Whilst anecdotal evidence exists that some cocoa varieties are more sun-tolerant than others, few experiments have dealt with this issue. Galyuon *et al.* (1996), in a limited study of photosynthetic rate in clones and seedlings, found no interaction between shade and genotype. Nevertheless, further studies of this sort are needed to broaden our understanding of whether differences in light tolerance exist amongst the germplasm.

Conclusion

This review has demonstrated how a physiological characterisation of cocoa germplasm can provide information that is both useful and relevant to the breeder, and that the techniques used to carry out such evaluation do not always need to be complex or costly. The incorporation of evaluations into established clonal trials can assist in the selection of desirable characteristics for yield enhancement. Of particular interest would be the physiological characterisation of geographically replicated clonal trials such as that established through the CFC/ICCO/IPGRI project on germplasm enhancement and evaluation (Eskes *et al.* 2000). Such an evaluation would give a greater insight into the interaction between genotype and environment.

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Genetic Effects of Inter-tree Competition in Mixed Cocoa Stands on Yield, Vigour and Cropping Efficiency

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Abstract

A method developed to assess family competition effects (partner effects) in variety trials was applied to the cocoa tree. The study was conducted in a hybrid trial, involving twelve families of 50 trees each planted in a totally randomised single-tree plot design, at a density of 1,667 trees ha^{-1}. The trial was thinned 10 years after planting, at a rate of two out of four rows. Competition was studied with reference to juvenile and adult vegetative vigour, to periodic and cumulative yields (number of pods, potential total pod weight and average weight of one pod), and the yield x vigour ratio or "cropping efficiency" (total pod weight divided by the trunk cross-section).

At the end of the trial, after thirteen years of monitoring, competition effects were revealed which explained 8 to 10 % of the residual variance after removal of the hybrid and micro-environmental effects. The competition effects, which were detectable by 18 months after planting for vigour, occurred earlier than generally acknowledged. Competition effects for yield increased with age of the plantation and decreased drastically after thinning at 10 years. Under the trial conditions, the families could be classified as "aggressive", "stimulating" or "passive" in relation to their neighbours. Vegetative vigour (trunk cross-sectional area) explained up to 34 % of the competition effects affecting vegetative development- total pod production and total pod weight over the period concerned. The partner effects on the production variables could never be explained by any of the production variables themselves, hence it should be possible to select non-aggressive high-yielding families. No significant partner effect was observed for average pod weight.

The improvement of the cropping efficiency, and more generally management of vegetative vigour, can follow genetic and plant husbandry pathways and these are considered in the Discussion. The genetic pathway entails the selection of hybrid or clonal material with low vigour and/or the search for dwarfing rootstocks. The plant husbandry pathway involves developing designs and techniques (such as thinning, pruning, *etc*...), which might decrease the competition effects between trees in a given plot situation.

Introduction

The planting densities currently adopted for cocoa cultivation usually vary from 1,111 to 1,667 trees ha^{-1}, corresponding to a spacing of 3 x 3m to 3 x 2m. Within three years, at those densities, the rapid formation of a continuous canopy helps to limit weed development and attacks of certain insects. However, competition effects between trees are commonly seen, such as the greater yields of border trees, which is a classic phenomenon in perennial crops (Corley 1977), or the increased proportion of pods in the canopy rather than on the trunks. Lachenaud & Oliver (1998) revealed the extent of such competition by thinning plots (reducing the total number of trees ha^{-1}), leading to a rapid increase in average productivity per tree by as much as five-fold. Also, in Côte d'Ivoire, such thinning techniques were used as a first step in regenerating old cocoa stands.

As vegetative vigour is often considered to be correlated with varietal competition effects, breeders use a composite selection criterion incorporating potential yield and vegetative bulk, the yield by vigour ratio, or 'cropping efficiency' (CE). However, few investigations have been made of competition in cocoa

cultivation, and the only practical results confirm the importance of borders in breeding trials (Glendinning & Vernon 1965; Lockwood & Martin 1976). In cocoa breeding, precise knowledge of competition effects would enable more reliable estimation of the genetic value of the varieties tested, and closer matching of varieties to various possible agronomic uses: semi-extensive smallholdings, or high density plantations, for example.

In coffee tree breeding, a method was proposed that enables the competition effects of each variety to be quantified, without using a co-variable linked *a priori* to those effects (Montagnon *et al.* 2001).The variables that most effectively explain competition effects can be determined *a posteriori*. The method was applied to cocoa, in a hybrid trial planted in French Guiana and monitored for 13 years.

Study carried out on competition effects

Methods applied

The study was conducted in a hybrid trial, involving twelve families of 50 trees in a totally randomised single-tree plot design, at a density of 1,667 trees ha^{-1} (3m x 2m). An additional family was created, which consisted of trees of uncertain genetic origin (off-types and replacement trees planted two years after the plot was set up). Moreover, the empty spaces left by dead trees were considered as a family (empty plots); the influence of missing trees over their neighbours could thus be studied (Lachenaud & Montagnon 2002).

The trial was thinned at 10 years, at a rate of two out of four rows, resulting in a final density of 833 trees ha^{-1} and a spacing of (3 m + 9 m) x 2 m. Two full harvesting seasons were monitored in the new design to study the effect of thinning on hybrid yields (Lachenaud & Oliver 1998). Competition was studied with reference to juvenile and adult vegetative vigour, and to periodic and cumulative yields (number of pods, potential total pod weight, average weight of one pod, and the yield : vigour ratio).

Vegetative vigour was quantified by recording trunk diameter 15 cm from the ground, after 4 and 18 months in the field, and girth 50 cm from the ground at 10 and 12.5 years, before and after thinning. These basic observations were used to calculate the following variables: increase in section between 4 and 18 months in the field, sections just before and 2.5 years after thinning, and the corresponding increase in section.

For yield, the following were recorded at each harvest: the number and weight of healthy pods, and the number of rotten pods. These data were used to determine the following, over variable durations: total number of pods, average weight of one pod, potential weight of all pods including rotten pods (potential yield).

Cropping efficiency was quantified by: CE_{93} = accumulated potential yield / 10 years trunk section (at the time of thinning), or CE_{96} = 2 years potential yield/ increase in trunk section, after thinning.

The analytical methodology for varietal competition effects was described in detail by Montagnon *et al.* (2001). In short, it includes i) a correction of the data by the Papadakis analysis of covariance in order to take into account the environmental effect in the vicinity of each tree and ii) to study competition between trees using the corrected data.

The basic model of the hybrid trial was: $X_{lt} = m + a_{v(l,t)} + E_{lt}$, where

X_{lt} = performance of the tree whose position is (l,t), l = line number and
t = tree number,
m = total mean of the trial,
v(lt) = hybrid to which the tree at position (l,t) belongs,
$a_{v(l,t)}$= effect of hybrid *v(l,t),*
E_{lt} = residual value of the tree.

A Papadakis analysis of co-variance was then run using the mean of the E values of the eight neighbours of (*l,t*) as the co-variable: if the co-variable had a significant effect, the data were corrected.

For each hybrid k, the existence of an additive competition effect C_k was postulated, which might modify the performance of the nearest neighbours of a tree belonging to hybrid k. The residual value ε_{lt} (after a possible Papadakis'correction) of the t [th] tree in the l [th] row was then modelled as: $\varepsilon_{lt} = C_{v(l,t-1)} + C_{v(l,t+1)} + \xi_{lt}$, where

$C_{v(l,t-1)}$, $C_{v(l,t+1)}$, are the additive competition effects of the hybrids to which the previous and the next tree in the same row belong, and

ξ_{lt} = error term (random effect, explained neither by environmental quality in the vicinity of the tree, nor by competition from the neighbouring trees).

The *K* parameters $C_1, \ldots, C_K$ could then be estimated as the coefficients of the multiple linear regression of the residuals on *K* variables. To ensure a unique solution, the constraint $\sum_{k=1}^{k=K} C_k = 0$ was added to the model (the departure of C_k from 0 was tested by a t-test on the regression coefficients).

C_k is defined as the "competition effect" of hybrid k, and has also been called the "partner effect" (Gallais 1975). If hybrid k was aggressive, *i.e.* a strong competitor unfavourable to its neighbours, then C_k was negative. Conversely, if hybrid k was stimulating, *i.e.* a weak competitor favourable to its neighbours, C_k was positive.

Once the competition effects of all the hybrids had been estimated, vigour traits or other variables that accounted for this variation were sought. For that purpose, a multiple linear regression was carried out, using the hybrid mean of each trait to explain the competition effect C. The 'stepwise' method was used and variables entered into the model if they were significant at the 0.05 level or below.

Results

In the trial, effects due to micro-environmental variations were slight and only occurred during the plot installation phase. They were not detected thereafter, except in a single case, where they were virtually negligible. This is a classic result in cocoa and coffee experiments (Paulin *et al.* 1993, Montagnon *et al.* 2001). However, competition effects were generalised and occurred as early as at 18 months after planting, well before the canopies closed. They explained from 4 % to 5 % of the residual variance in the first 5 years and 8 % to 10 % thereafter. Significant aggressive and stimulating families were identified.

Competition for *juvenile vegetative* vigour was revealed for stem diameter at 18 months and the increase in trunk section between 4 and 18 months. However, competition could not be predicted from family vigour *per se* assessed by the three available vigour variables. For instance, a family showed considerable aggressiveness towards its immediate neighbours, whereas it was a medium-vigour family at a young age and a low vigour family thereafter. In fact, the aggressiveness of this family (Amelonado control) may have been due to its attractiveness to insects (or diseases) to the detriment of the early development of its neighbours. Competition was weak for adult vigour: no significant partner effect at 10 years, and only two at 12.5 years (2.5 years after thinning), including the empty plots.

Prior to thinning, competition for *yield* was generalised: an increasing number of families showed a significant C value as the trees aged and an increasing percentage of residual variation was explained. At 10 years, 6 and 5 out of 12 families had a significant C value for the number of pods and the potential total weight of pods, respectively (and for these two traits, the empty plots also had a

significant C value). The average trunk cross-section of the trees in 1993 explained 34 % of the variation in partner effects on the 1992-93 pod production (in number and weight) in our trial (see Table 1). The effects of competition were most obvious, as shown by the more significant C values, when the number of pods harvested was considered rather than their total weight.

Two years after thinning, competition was no longer detectable. That observation confirmed the merits of this practice in decreasing inter-varietal competition (Lachenaud & Oliver 1998).

No significant partner effect was observed for average pod weight.

Discussion

Theoretical conclusions (Demarly 1975) or practical observations on other perennial crops such as apple (Parry 1978) and oil palm (Nouy *et al.* 1990) have shown that competition initially affects yield in terms of number of flowers and fruits, then fruit size, and only later vegetative vigour. This seemed also the case in our trial, since we observed strong competition effects for production while almost no competition was detected for adult vigour.

Competition had a differential effect on vigour and yield and was thus expected to influence the cropping efficiency (CE). In fact, four families (and also "empty plots") showed a significant partner effect for CE at the end of the hybrid trial (1993), but after thinning only one family did so and no empty plot effect was noted. Similar effects have been reported in temperate fruit trees (Parry 1978), where, for the same cultivar, CE diminished as competition effects developed. In our study, families that most benefited from thinning through an increase in CE were also among the most "stimulating" before thinning. However, in our study, no correlations were noted between the C values for CE before and after thinning.

The large proportion of the variation in partner effects explained by the trunk cross-section of the trees in 1993 (Table 1) justifies, with hindsight, the fact that cocoa breeders take the section into account as a compensation parameter assumed to represent the competitive abilities of a tree (Lotodé & Lachenaud 1988). However, the section in 1993, at the end of the hybrid trial, did not explain family partner effects for cumulated production, though it did so for the 1992-93 crop. It might thus be useful to multiply tree size (trunk growth, canopy size,..) measurements, for example every two years, or to consider other variables that may be involved in competition (root exudates, leaf volatile substances, insect attractiveness, *etc.*).

The competition effects affecting the yield variables were never explained by the yield variables themselves, which is favourable for selecting productive, but non-aggressive, varieties. Due consideration of the identified partner effects could be of real value in selecting varieties adapted to various types of crop management. For instance, under semi-extensive conditions, such as those in traditional medium-density smallholdings (1,000-1,500 trees ha^{-1}), varieties with a high cropping efficiency, but with low aggressiveness are sought. In that case, families with a high yield : vigour ratio and a non-significant partner effect would be the most appropriate. For high-density plots (> 1,500 trees ha^{-1}), such as "fruit hedge" designs, aggressive families ($C < 0$) should be ruled out. In that case, only families which had a satisfactory cropping efficiency, with low or moderate vigour and a "passive" or stimulating effect should be retained. Furthermore, stimulating but low-yielding families could also be tested under these conditions.

Table 1. Some examples of family partner effects (C) for total number of pods (T), potential yield (Pot, in kg of pods) and for cropping efficiency (CE, in kg of pods per cm ^{2})

Family	T_{92-93}		Pot_{92-93}		Pot_{86-93}		CE_{93}		CE_{96}	
	C	Mean	C	Mean	C	Mean	C	Mean	C	Mean
1	-0.0	57.6	-0.0	24.2	1.7	60.3	0.03	0.43	0.11	2.36
2	-8.7	50.8	-3.5 *	24.4	-3.7	62.9	-0.00	0.32	-0.17	0.78
3	11.9 *	10.7	4.7 *	4.4	8.8 *	14.5	0.05 *	0.16	-0.15	0.57
4	-0.1	31.1	-0.5	12.2	-0.2	33.2	0.01	0.30	0.05	0.69
5	-3.6	33.4	-1.1	15.0	-2.6	36.7	-0.01	0.31	-0.08	0.82
6	9.5 **	9.7	4.3 *	3.2	9.3 **	9.3	0.06 **	0.12	0.93 ***	0.53
7	13.2 **	34.7	5.0 **	13.6	9.3 **	30.6	0.04 *	0.22	0.27	0.66
8	-9.1	91.2	-3.0	37.7	-7.0 *	81.0	-0.04	0.45	0.22	1.58
9	15.2 ***	12.6	4.6 **	5.7	4.9	20.5	-0.00	0.22	-0.20	0.65
10	-9.4 *	41.7	-3.2	14.4	-5.0	26.4	-0.04	0.19	0.02	0.59
11	0.2	5.2	-0.1	2.6	-1.5	10.2	-0.00	0.14	0.34	0.34
12	-8.4	13.4	-3.1	6.3	-7.7 *	19.4	-0.04	0.19	-0.05	0.63
13	-18.9 *	35.1	-7.8 *	17.6	-13.6 *	40.4	-0.10 *	0.28	-0.51	1.30
Empty plots	8.1 *		3.6 *		7.4 **		0.04 *		-0.11	
% V^2 (1)	7.9		7.8		7.4		6.1		9.2	
R^2 S_{93} (2)	0.34		0.34							

*, **, *** : significant at the 5 , 1 and 0.1 % levels of probability.
(1) % of residual variance explained by competition effects
(2) % of variance in C values explained by progeny mean trunk section in 1993

Perspectives for further research

Taking competition into account in tree cultivation involves an adaptation either of densities to varieties or variety vigour to density. Both options can be considered in cocoa cultivation. In the second case, the described method quantifies numerous significant partner effects, on a family level, for vegetative development and yield variables. Our results confirm the merits of thinning cocoa plantings after approximately ten years: indeed, the production increase seen after thinning (Lachenaud & Oliver 1998) seemed to be linked to a reduction in negative partner effects, hence more effective management of competition between trees.

Nevertheless, additional trials are necessary to determine the behaviour of families classed as "aggressive" or "stimulating". What would happen if two aggressive families were mixed in the same plot? Or two stimulating families? Glendinning & Vernon (1965) reported in cocoa trees that the dominated family in a high competition situation was the one with the highest yields when no competition existed.

In cocoa agronomy, the degree and harmfulness of competition necessitates the development of ways of managing the yield : vigour ratio that are more elaborate and efficient than the few methods currently used, such as pruning to reduce canopy size and height. This requires that cocoa researchers use their skills to establish crop

management sequences based on physiological justifications. In particular, it first seems necessary to select high-yielding varieties with low competitiveness. In this respect, priority should be given to studying the yield : vigour ratio and, more specifically, the harvest index (HI), which is the harvested proportion of the (net) dry matter produced.

In fact, it is a matter of encouraging reproductive phenomena, to the "detriment" of vegetative development. Basic physiological studies should make it possible to identify the phenomena involved and, possibly, reveal new early selection criteria. Nevertheless, a clearer understanding needs to be acquired of the yield elaboration mechanism and associated processes (in particular compensation between various fruiting factors and influences between organs) and of correlations (genetic and environmental) between yield components ("selection traits" or "selection criteria"). Such management of the yield : vigour ratio might possibly be achieved by two different approaches: the genetic pathway and the plant husbandry pathway.

Genetic pathway mechanism

The aim is to obtain germplasm that is more appropriate for the high densities used and necessary in practice (> 1,000 trees ha^{-1}), either by selecting and creating hybrid material (seedlings) or clones with low vigour, high CE and non-aggressive characters, or by using dwarfing rootstocks enabling high (or even very high) densities to be used.

Selection for low vegetative vigour:

Index-based selection would assign the main weight to the yield : vigour ratio, which is already done empirically in some cases. This aspect of work should involve the following points (non-exhaustive list):

- A study of variations in CE over successive cropping years (it should not be considered as a genetic "constant"):
  - depending on density and competition,
  - which yield components are affected?
  - determination of the genetic share of variation and classification of hybrid families as aggressive, indifferent, and stimulating for others (Lachenaud & Montagnon 2002).
- A study of the feasibility of basing selection on CE (in particular, result stability depending on different plot and local environments),
- Selection and genetic improvement based on that ratio.

Some existing trials could already throw light on some of these points, including two "low vigour" trials that were planted in Côte d'Ivoire in 1987 to compare seven hybrid progenies at two densities (1,600 and 1,333 trees ha^{-1}). Results from these trials, which should be available now, would need to be analysed according to the different aspects mentioned above.

Selection of dwarf cultivars:

- Research on and establishment of dwarfing rootstock trials,
- Studies on rootstock-scion relations and on the wider issue of grafting problems (effect of grafting on various criteria, including overall yield, yield components, and quality criteria).

Plant husbandry pathway

This pathway involves the search for and development of techniques and/or procedures to make it possible to increase the yield : vigour ratio in a given plot situation. In conventional cocoa growing, for a given genotype, density is known to be a paramount, if not the main, yield factor (and component), but the optimum value

evolves in line with the age of the plots. One solution would therefore lie in adopting an "evolving density".

Procedures enabling application of the "evolving density" concept:
A study of procedures making it possible to effectively manipulate the yield : vigour ratio of cocoa trees by progressive thinning, which is common in tree cultivation and forestry (Lachenaud 2002; Lachenaud & Montagnon 2002).

Pruning and fertilizers:
Studies on various types of pruning (maintenance and formation pruning) and how they affect the yield : vigour ratio. Likewise, studies on how various nutrients and trace elements affect that ratio.

Physiological aspects:
Variations seen in the "harvest index" can be explained by studying the distribution (or translocation) of assimilates between the different "sinks" (and "sources") (Daymond *et al.* 2002). The ultimate aim would be to identify physiological mechanisms (and the factors on which they depend) involved in the expression and variation of HI and CE (why do certain clones perform well, *e.g.* Upper Amazon and Guianan types– and others not so, *e.g.* Trinitarios) and possibly determine new earlier "predictive" selection criteria.

It would also be necessary to ascertain the different successive reproductive strategies of cocoa trees in a situation of increasing competition: which "sinks" are affected, to what degree are they affected, and in what sequences? As we have already seen, it is known (theoretically and in practice in the apple tree and oil palm) that fruit production is the first to be affected by the development of competition, first and foremost in terms of numbers (of flowers, then set flowers, then fruits), followed by the average fruit size. Thereafter, vegetative development can be affected. These first observations on cocoa seem to confirm these points.

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Enhancing Harvest Index in Temperate Fruit Tree Crops Through The Use of Dwarfing Rootstocks

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Abstract

The aim of this paper is to evaluate information relevant to the use and development of rootstocks from temperate fruit trees (particularly apple, pear and cherry) as an effective means of enhancing harvest index (*e.g.* weight of useable fruit yield relative to total plant weight). The intention is to provide information to facilitate exploration and discussion of which scientific approach(es) might best suit the future development of cocoa growing.

The grafting of clonal scions onto rootstocks is an ancient practice initiated to obviate problems associated with not being able to obtain trueness-to-type from seedlings and limitations in the ability to root scions directly for vegetative propagation. Subsequently, it became apparent that many features of the scion could be beneficially influenced. In particular, these included increases in harvest index, precocity (reducing the period of the juvenile phase), yield (productivity), fruit size and quality, tolerance to climatic stress, pest and disease resistance, as well as the ability to control tree size (dwarfing). Direct rootstock advantages, such as increased anchorage and improved uptake of water and nutrients may also improve the harvest index. Crops can also be grown at higher densities and this can increase production per unit land area.

For temperate tree crops, control of size has become essential for economic fruit production and this is now achieved primarily by scion grafting or budding onto dwarfing rootstocks. Trees of reduced size are much easier and therefore less expensive to manage (prune, spray and harvest) compared to traditional, large trees. Dwarfed trees provide efficient targets for the application of pesticides, and reduce the impacts of unwanted chemical spray drift and environmental contamination. Current knowledge of the mechanisms by which apple rootstocks influence shoot behaviour is presented with a view to establishing relevance and potential exploitation in other crops, such as cocoa.

Alternative approaches to the use of dwarfing rootstocks, as effective means of controlling shoot growth, are also outlined, including the use of chemical plant growth regulators, restriction of root growth and withholding irrigation at critical times in the growing season.

Introduction

The aims of this paper are to describe the development, the benefits and the mechanisms by which dwarfing rootstocks in temperate fruit crops, such as apple, have been used to enhance fruit production. The intentions are to determine relevance and feasibility of these studies to the further development of cocoa (*Theobroma cacao L.*) production. In an earlier attempt to utilise ideas and approaches from temperate fruit production, Posnette (1982) described the intensive high-density apple planting systems and more specifically their relevance to cocoa. He concluded for cocoa that selection for either precocious-bearing clones with moderate vigour, or dwarfing rootstocks was required. Subsequently, Tukey (1993) also strongly supported the modern apple orchard growing systems, as a way to adapt cocoa plantings to modify plant architecture and enhance field production. Tukey (1993) also highlighted the need for "an active research and development programme" to identify dwarf cocoa genotypes" with a view to determining their potential to control cocoa tree size. This conclusion was based on the need for economic benefits to be achieved through increases in mechanisation in a crop where growth is controlled and the efficiency of production enhanced. This is certainly the approach that has driven temperate fruit

production over the last 50 years, from a low planting density (250 trees per hectare) utilising large difficult-to-manage trees, to high-density spacing (2000 to 5000 trees per hectare) with intensively managed trees. It is difficult to imagine how this could have been achieved without the use of dwarfing rootstocks.

Discovery and development of dwarfing apple rootstocks: a brief history

Malus is a diverse genus with around 25 to 30 species with further sub-species in the crab apple group. The common apple, as we now know it, has not evolved naturally and contains the germplasm of many wild progenitors. The genus is now found throughout North America and Eurasia, but it is suggested that the primary centre of origin spanned Asia Minor to the western edges of China (Janick *et al.* 1996). The cultivation of apple extends into prehistory with evidence of carbonised fruits dating back to 6500 BC in Anatolia (Juniper *et al.* 2001). Selected apples and pears spread from early civilizations in the Fertile Crescent, in Iran, prior to 2000 BC, when apple is known to have reached Palestine and subsequently Egypt, in the time of Rameses II between 1293 and 1235 BC (Jackson 2003). Alexander the Great is listed as one of the many early collectors of plant material from Asia Minor. Theophrastus, a pupil of Aristotle, mentioned the use of dwarf apple in his writings. The Roman Empire was also well aware of the value of apple, the writer Pliny has been accredited with some of the earliest guidelines regarding economic apple production. There are also various Roman scripts describing different types of grafting, which have changed little in the intervening centuries. The construction of stores and the process of apple storage was also a practice used by the Romans (Varro 116-27 BC).

The custom of grafting fruit trees onto a rootstock is very ancient. The need for grafting came about because selected scions types, which were valued for their fruit quality and desirable characteristics, could not be raised true-to-type from seed. Apple scion cultivars are also extremely difficult to produce vegetatively because of the difficulty in getting them to root using the traditional approaches of layering/stooling. Micropropagation has been used to aid rooting but may create a whole new set of problems associated with weaning and transplantation. Seedlings also show juvenile characteristics, which extends the time period before reproductive maturity, cropping and economic return. To overcome these problems, scions were propagated by grafting or budding onto a rootstock. Initially, this approach had little to do with the benefits associated with the dwarfing of scions or any desire to alter scion performance characteristics. Rootstocks were produced asexually in stoolbeds or by hardwood cuttings.

The use of the composite tree (scion and rootstock) approach to adapt fruit production to specific regions, climates and tolerance to pests has driven the process of grafting from its origins. This is clearly evident in the writings of the poet Virgil, where he gives importance to the selection of material for grafting (as quoted by Hatton 1917). Indeed, the selection of rootstock is the single dominant factor in the development of the modern apple orchard. It is interesting to note that Hatton (1917) in his early publications suggested that little advance had been made beyond the rudimentary advice available in publications such as the 'The Complete Planter and Cyderist' (1685). Hatton also suggested that this was due to a "neglect of studying the tree".

Fruit growers in the 16th century extensively used a rootstock called 'Paradise' (first recorded mention in 1536) because of its precocity (crops earlier in the life of the tree) and uniformity. 'Paradise' did, however, dwarf the scion grafted onto it. Prior to this, English growers used rootstocks raised from seed, particularly those for the production of cider and in some cases seedlings of *M. sylvestris* were used. The 'French Paradise' form is believed to have originated in Armenia as *M. pumila*, or as a cross between *M. pumila* and *M. sylvestris*. It was this material that was initially introduced into southern England and propagated in Kentish nurseries. Unfortunately,

due to introductions and the inclusion of the less dwarfing 'Doucin' (or 'English Paradise') rootstocks over many years, it became apparent that there were numerous 'types' regarded as 'Paradise' rootstock. The descriptive information about the performance of these types was very limited. By the 1800's there were 14, apparently different, types of Paradise rootstock (Rivers 1865). This situation induced considerable confusion over identity and trueness to type (see Hatton 1917, for further details).

After the formation of the East Malling fruit research station in 1913, in Kent, researchers Wellington and Hatton planned to address this classification problem. Hatton initially obtained more than 70 'types' of rootstocks from over 35 sources, which he found to be either incorrectly named, or included in the collection more than once. He then embarked on a comparative botanical/anatomical descriptive reclassification of these major rootstocks (Hatton 1917). The descriptions of the first nine (I to IX) were subsequently added to, in stages, to give 24 (XXIV) rootstocks (Hatton 1917). Initially, they were labelled with the classification tag EM, which was subsequently replaced with the letter M. The dwarfing M.9 is currently the most widely used apple rootstock in the world. Other rootstocks were added to this list from breeding selections made at East Malling (M.25 and M.26). At this time The John Innes Institute, at Merton in the UK, produced crosses using some East Malling types to introduce resistance to woolly apple aphid for use in New Zealand and Australia. Further collaborative breeding between the John Innes Institute and the East Malling Research Station produced the M.M. series of rootstocks with aphid resistance (Jackson 2003).

Benefits of using rootstocks to influence shoot behaviour

The original reason for using rootstocks was to propagate valued scions that could not be self-rooted. So the initial primary benefit of using a rootstock was to be able to propagate scions vegetatively. Such an approach would provide sufficient material to enable orchards of a single cultivar to be planted. This was the first step in the growth of commercial fruit production on a large scale. Also apparent from the process of grafting the rootstock and scion was an increase in precocity (earlier reproductive development). The considerable advantage in the use of the Paradise rootstock, and the reason why it was used for over 300 years, was that it also dwarfed the scion that was grafted on to it. The key initial feature of the rootstock scion-grafting interaction was that the efficiency with which dry matter could be partitioned into fruit production could be increased. A dwarfing rootstock increases the amount of dry matter allocated to fruit relative to the vegetative growth. In general terms dwarfing rootstocks have higher yield efficiencies, *i.e.* the crop weight per unit of tree trunk cross-sectional area, than non-dwarfing rootstocks. A point can however be reached where there is too much dwarfing effect: despite high fruit production efficiency per tree, the canopy size is restricted and the allotted space in the orchard is not filled and yield per unit land area is not maintained.

Clonal apple rootstocks also confer a number of other specific traits/characteristics, which include: resistance to insects and disease, tolerance to soil and climatic factors and anchorage and ease of propagation. Several rootstock effects have been shown to be unrelated to changes in vigour or carbon partitioning to dry matter alone. Rootstocks, for example, also influence the time of leaf senescence, leaf size, photosynthetic rate, flower morphology, flowering precocity, fruit set, apical dominance, tree shape, cambial differentiation, and the duration and rate of tree growth (Webster 1995).

Growth control

In several temperate tree fruits (apple, pear, plum and sweet cherry) the use of rootstocks has provided the principal method to control scion vigour. It should be noted, however, that rootstocks have never been effective in completely controlling vegetative growth. Shoot and root pruning, and chemical growth retardants have also been

required. Controlling scion vigour is becoming increasingly important as economic pressure reduces the viability of worldwide fruit production. Dwarfing enables tree management and harvesting to be carried out at ground level. In the past, larger trees required much more labour intensive systems to pick fruit and manage tree growth, along with increased applications of chemical to control pests and diseases. The dwarf tree provides the means by which dry matter production can be directed towards harvesteable yield, thereby reducing waste, with respect to unwanted vegetative growth and pruning costs. It also allows more trees to be planted per unit of land area and this can increase production. There are of course considerable economic implications for growing crops in high density planting, particularly those associated with planting costs.

Soil and climatic conditions

Rootstocks have also been used to provide tolerance to adverse climatic and soil conditions. Current limitations in soil-water availability and predicted likely impacts of climate change in some growing regions have stimulated research into the evaluation of rootstock drought tolerance (Rom and Carlson 1987, Fernandez *et al.* 1997, Atkinson *et al.* 1999 and 2000a). Similarly, the anaerobic conditions produced during flooding have become a more important issue, with respect to perennial crops that have to over-winter in waterlogged soils. Not all tree species show the same tolerance, for example peach and cherry are susceptible, grape is resistant and apple is intermediate. Since most commercial apple cultivars are grown on dwarfing or semi-dwarfing rootstocks, there is interest in identifying any differences in their sensitivity to flooding and examining the physiological and molecular bases for such differences. Previous work (Al-Husainy and Jackson 2000) has indicated that differences in tolerance do exist between apple rootstocks and that these may be linked to the propensity to form adventitious roots that partly replace the more deeply penetrating original roots which are damaged by the flooding. Removal of these new roots as they emerged increased greatly the loss of leaves during flooding and subsequent drainage and reduced survival (Al-Husainy and Jackson 2000). Adventitious roots may confer flooding tolerance by virtue of their extensive aerenchyma, which would facilitate the entry of air to re-oxygenate the rhizosphere. The flooding tolerance of M.M.106 has been attributed to its production of adventitious roots (Anderson *et al.* 1984). Alternatively, these new roots may restore root to shoot communication and export essential mineral ions and hormones to the shoot system. Exposure to water-logged soils for extended winter periods is a serious concern in regions where climate model scenarios predict climatic changes in the near future.

The early work of Hatton (1935) showed that non-dwarfing rootstock and scion composite trees produced larger root systems than dwarfing rootstocks. A positive linear relationship was apparent from these data, which showed that the mass of the root system and that of the above ground scion was in a ratio of 1 to 4. The expression of apple scion vigour has also been shown to be linearly correlated with the number of rootstock roots, when the same scion was grown on a range of rootstocks of different vigour (Fernandez *et al.* 1995). This work describes, statistically, the relationships between rootstock vigour (root system size), scion vigour and yield. More recent work suggests however that root system size may not always be simply related to the capacity of the rootstocks to dwarf the scion (Atkinson *et al.* 1999). Rootstocks alone also influence the size of the root system and therefore the volume of soil exploited (Atkinson 1980, Atkinson *et al.* 1999). The root systems of apple trees and to some degree pear tend to be considered as sparse ranging from 0.8 to 23.8 cm of root per cm^2 of soil surface for apple and 7 to 69 for pear (Atkinson 1980). These figures translate into densities (cm of root per cm^3 of soil volume) of 0.01 to 0.20 and 0.12 to 0.56 for apple and pear, respectively.

There are also rootstock differences in the seasonal timing of root growth and both these factors influence the adaptability of a rootstock to different soil types. Atkinson (1980) suggests that it is, however, a misconception that dwarfing rootstocks,

such a M.9, root more shallowly than vigorous rootstocks. He concluded that rooting depth was a feature of soil type rather than rootstock.

Different rootstocks appear to show variable optimum temperatures at which maximum growth was evident (Rom and Carlson 1987). Low winter temperature has also been a critical factor in developing production in certain regions within Europe and North America and Canada. Freezing injury to roots is known to cause tree losses. Rootstocks show genetic variability in their resistance to winter injury (Quamme 1990). In many such cases the standard dwarfing apple rootstock M.9 was shown to be too sensitive to the sub-zero temperatures normal for these regions in the dormant season. Evaluation has enabled resistance to freezing temperatures to be assessed and advice given on the selection of rootstocks with improved tolerance (Quamme and Brownlee 1997). These authors and others have also reported that some rootstocks (Robusta 5 and crab apple rootstocks) can improve cold hardiness of grafted scions (Rollins *et al.* 1962). This may be achieved by the rootstock increasing the rate at which scion maturity is reached in the autumn, or delaying bud break in the spring (Rom and Carlson 1987).

Soil-borne pests and diseases

There are many pest and disease problems associated with fruit production in temperate regions. The most important diseases that affect apple rootstocks are fire blight, which is caused by the bacterium *Erwinia amylovora* (Burr) and crown (collar) rot, caused by *Phytophthora cactorum*. There is rootstock variability in resistance to key economic soil-borne diseases such as *Phytophthora* sp. and resistance to important pests such as woolly apple aphid (*Eriosoma lanigerum* Hausm.) This has been improved through the use of breeding programmes (Rom and Carlson 1987). In the case of woolly apple aphid, the cultivar 'Northern Spy' is resistant and has therefore been extensively used as a parent in breeding programmes, *i.e.* the resistant Malling-Merton (MM) rootstocks. There is, however, virtually no understanding how tolerances or resistances have been achieved. Rootstock variability in viral tolerance (*i.e.* tomato ring spot) has also been demonstrated (Rom and Carlson 1987).

Anchorage

Most if not all trees on dwarfing rootstocks require additional support, often afforded by stakes or an appropriate trellis. Support is required because high crop loads will damage the above ground framework of the tree and may also cause root breakage. It is often suggested that apple roots are brittle by nature and any movement of the scion can cause a loss of functional root. One of the major defects of M.9 is its poor anchorage (Webster 2002). This brittleness is suggested to be due to short fibres and a high proportion of root bark (relative to xylem) (Rom and Carlson 1987). As already suggested, apple rootstocks root sparsely and this can contribute to tree instability, but it remains somewhat unclear if there are actual rootstock vigour-related differences in rooting density which may influence anchorage (Atkinson 1980). Higher scion budding combined with deeper planting may allow more root system to develop on the longer rootstock shank and improve anchorage.

Understanding the mechanisms by which dwarfing rootstocks influence the behaviour of shoot

Despite many studies involving composite (shoots grafted onto roots) perennial plants, the mechanism(s) by which roots influence shoot vegetative growth and development, are not fully understood (Beakbane 1956; Tubbs 1973a and b; Lochard and Schneider 1981; Jones 1986; Atkinson and Else 2001).

Anatomy of the rootstock

There is considerable evidence (Beakbane and Thompson 1947; Simons 1986), with perennial fruit trees, that the root systems used in composite plants have quantitative differences in anatomical xylem structure and this can be linked with the potential of the root system to dwarf the shoot, perhaps by reducing stem water flow. Root systems that dwarf shoots have a low xylem to phloem ratio while the opposite is true for root systems that promote shoot growth which have xylem tissue with more and larger vessel elements (Beakbane and Thompson 1947). The consistency of this ratio has enabled it to be successfully used, at the seedling stage, in selection protocols for apple 'rootstock' (clonally produced root system) breeding programmes (Beakbane and Thompson 1947, Rogers and Beakbane 1957, Miller *et al.* 1961). Differences in the width of phloem sieve tubes have also been correlated with rootstock vigour in *Prunus mahaleb* (Misirli *et al.* 1996).

Potential for rootstock-derived differences in hydraulic capacity

Recently some attempts have been made to quantify the amount of functional xylem area in stem sections by staining with aqueous safranin solution (Atkinson and Else 2001; Atkinson *et al.* 2003). There was a reduced amount of xylem area stained in the scion grafted to a dwarfing M.27 rootstock; this suggests a reduction in functional xylem area compared to M.M.106. Direct measurements of stem hydraulic conductance revealed that the vigour of the rootstock onto which a scion was grafted influenced the scion hydraulic capacity. This occurred independently of conductance changing simply in relation to rootstock-induced changes in stem diameter or supported leaf area. Measurements of stem hydraulics, across a combined series of resistances, from the rootstock shank, through the graft union, to the scion, showed that conductance was related to rootstock vigour (Atkinson *et al.* 2003). This was is in agreement with the studies that quantified the movement of aqueous safranin solution across the graft union and those of Warne and Raby (1938). Vigorous rootstocks had the highest total conductance. When the graft union resistances were calculated the largest factor contributing to this variation between rootstocks was the graft union itself. These results show that the hydraulic resistance of an M.27 dwarfing rootstock graft union was much greater than that of the vigorous rootstock M.M.106. The semi-dwarfing rootstock M.9 was of an intermediate value between these two rootstocks. The measurements used to make these calculations were normalised to account for rootstock differences in stem cross-sectional area, but differences in conductance existed between rootstocks on a unit stem cross-sectional area basis. Differences in whole intact stem conductance, and therefore, sap flow in the xylem between rootstocks, may be however, less evident as the graft union cross-sectional area increases with dwarfing rootstocks. Such an increase in the graft union area may be a mechanism by which dwarfing rootstocks attempt to overcome the hydraulic limitation imposed by the graft union tissue and its abnormal xylem anatomy.

Changes in rootstock and scion transport capacity

Changes in cellular graft anatomy are often evident when perennial woody species are grafted onto roots (rootstock) that restrict the vegetative growth of the shoot (scion), *i.e.* a dwarfing rootstock (Warne and Raby 1938; Simons 1986; Soumelidou *et al.* 1994a). It has been suggested that the graft tissue itself has a role in influencing vegetative shoot growth by restricting water flow from the root to the shoot or by removing substances, particularly minerals and plant growth regulators (*i.e.* cytokinins), in the xylem stream (Knight 1926; Jones 1974; Jones 1984). The restriction of water flow is entirely consistent with the anatomical changes associated with graft tissues and the different degrees of shoot dwarfism shown in composite plants (Mosse 1962; Simons 1986; Soumelidou *et al.* 1994a). These anatomical changes may be due to limitations in polar auxin transport across the graft and its accumulation at the graft (Soumelidou *et al.* 1994b). Auxin is a key leaf-derived regulator of xylem cell differentiation and division

within the cambial zone and an initiator of vascular redifferentiation across the graft union (Parkinson and Yeoman 1982; Hess and Sachs 1972; Savidge 1988). A similar argument has been made for the differentiation of water transporting tracheids in graft tissue of *Picea sitchensis* (Weatherhead and Barnett 1986). Much more precise tissue specific information is now evident, linking the gradient of cellular auxin concentration to developing cell types within differentiating xylem (Uggla *et al.* 1996).

Differences in the transport capacity of grafted tissue may also explain how budding height influences scion dwarfing. It is well documented that the height at which a scion is budded *i.e.* the length of the dwarfing rootstock 'shank', has a positive effect on the extent to which the scion is dwarfed (Van Oosten 1978).

Hormonal aspects of rootstock-derived control of scion growth and development

Recent experiments have highlighted how roots respond to the amount of available soil water, and communicate with the shoot to affect a co-ordinated response, using abscisic acid (ABA) in the transpiration stream (Davies and Zhang 1991). When the ABA signal arrives in the leaf, the aerial control of water use efficiency is affected, by alteration of stomatal conductance. Leaf growth may also be affected. The ability of semi-invigorating and dwarfing rootstocks to export ABA in the xylem sap to the shoots is currently being investigated (Else and Atkinson, unpublished).

There is also strong evidence to show that cytokinins are synthesised in the roots and transported to the shoot via the xylem, in the transpiration stream (Wareing 1977). In the shoot, cytokinins can influence growth, photosynthesis and leaf senescence. Root pruning has been shown to have different, apparently species-dependent effects on the shoot cytokinin level. In apple, root pruning appears to reduce shoot cytokinin levels that in turn influence flowering, pre-harvest drop and shoot growth. Work at HRI-East Malling has shown recently that cytokinin concentration in xylem sap increased with the increasing vigour imparted by the rootstock and this accounted for an increase in shoot cytokinin content (Kamboj *et al.* 1999). In experiments elsewhere, drought stress resulted in a large reduction in shoot cytokinin, despite an increase in root production, which implies that transport was inhibited, but it remains unclear how (Bano *et al.* 1993).

Roots have also been suggested as the sites for the synthesis of gibberellins (GA); they may also be sites involved in the inter-conversion of shoot-derived GA. Richards *et al.* (1986) using radioactively labelled gibberellin (GA_4) suggested that GAs were involved in the dwarfing response mechanism. However, their measurement approach can be criticised as they only reported GA-like activity declining in dwarfing interstocks and the methodology used was one of hormone application rather than direct *in planta* measurement. Adverse conditions in the rhizosphere have been shown to affect the amount of GA exported from the root. For example, low temperatures reduce GA in root sap and this, in turn, reduces shoot length (Steffens and Hedden 1992). Auxin can also be derived from roots; the root cap appears to be the most likely site of synthesis. IAA levels have been shown to change with the developmental age of roots and with other factors such as high mechanical soil impedance.

Hormonal aspects of scion shoot derived control of rootstock growth and development

Leaf-derived auxin appears to be the most potent scion-derived signalling system that influences root behaviour (Aloni 1987). The role of auxin in initiating cell wall loosening and cell extensibility has long been known, as has its ability to be transported basipetally in the phloem and by the polar auxin transport pathway. New evidence suggests that auxin movement is predominantly in parenchymatous and cambial cells (proto-xylem and –phloem), and it can move acropetally as well. High accumulations of auxin favour differentiation into xylem elements. Conversely, low concentrations of auxin promote phloem development. The higher ratio of xylem to phloem tissue in

rootstocks as vigour increases may be a reflection of differences in hormone action and/or transport. The available evidence suggest that the leaf canopy (leaf area), as a source of auxin, determines the amount of xylem that develops (Doley and Letyon 1968; Hess and Sachs 1972). This functional/structural interaction can be supported and described by an allometric relationship between stem area and various whole canopy processes, like reproductive potential and transpiration (Savidge 1988; Niklas 1993).

Recent work at HRI-East Malling suggests that the level of IAA in the bark of different apple rootstocks was fairly constant, and that the rate of transport changes (Kamboj *et al.* 1997). However, this was not the case with dwarfing citrus rootstocks (Bester and Rabe 1996). In dwarfing apple rootstocks the movement of IAA appears to be slower on a stem cross-sectional area basis, compared to invigorating rootstocks, despite there being relatively more transport tissue (phloem). Earlier work has shown that a higher phloem : xylem ratio was found to be associated with dwarfing, compared to vigorous rootstocks (Beakbane and Thompson 1947). More direct support for the role of carbohydrates in the shoot has been forthcoming from studies involving auxin. Some anatomical characteristics of dwarfing rootstocks have been attributed to a higher ratio of carbohydrate to auxin than evident with vigorous rootstocks. Dwarfing rootstocks contain more starch reserves and the accumulation of leaf starch is known to down regulate photosynthesis.

Looking for alternatives to the use of rootstocks?

Reduced shoot growth on apple trees can also be achieved using other complementary management strategies to dwarfing rootstocks (Webster 1995). The most popular management techniques of this type involve the pruning and/or training of extension shoots. However, treatment with chemical plant growth regulators (Quinlan 1981) or withholding supplies of supplementary irrigation at critical times in the growing season (Chalmers *et al.* 1981 and 1986), have also proved effective means of shoot growth control in some apple-producing areas of the world. With a few scion cultivars, such as Red Delicious, compact or spur type mutant clones provide some of the necessary control of vigour

Growing scions on their own roots

Recent research efforts have now facilitated the culture of many scions vegetatively without the need to bud or graft onto rootstocks, (Webster 1995). Micropropagation has enabled most scions to be propagated on their own roots, but the approach is not without problems. These include a failure to establish when planted out and 'false juvenility', a condition induced during culture. An extension of the juvenility period is not desirable and can occur with alterations in branching architecture, along with burrknotting and suckering (Webster 2002). Furthermore, self-rooted scions are generally more vigorous than when grown on dwarfing rootstocks. Self-rooted trees will need to crop as well as those on rootstocks and their production costs need to be viable; current economics suggest that they are not going to be cheaper to produce than those on conventional rootstocks.

Compact mutant forms of commercial scions

The availability of certain compact scion mutants of several apple cultivars ('McIntosh' and 'Delicious') have been particularly useful in producing trees showing reduced vegetative growth without yield loss (Tukey and Ballard 1969; Lord, *et al.* 1980). The yield, and in some cases the fruit quality, has not reached sufficiently high enough standards with many spur or compact types, *i.e.* Cox's Orange Pippin, Granny Smith and Bramley's Seedling and the sweet cherry varieties Van and Stella. It is currently not understood why some of these scion mutants with reduced vigour have low yield

efficiency. Some are also unstable due to the chimaeral nature of the mutation. The effects of these mutations are to bring about internode shortening, frequently by inhibition of gibberellin biosynthesis. This is not the same mechanism by which dwarfing rootstocks influence scion growth.

Chemical control of shoot growth

There are a number of chemicals (daminozide 'Alar', paclobutrazol, 'Cultar', CCC, 'Cyclocel'), which have been used with some success to restrict shoot growth (Quinlan 1981, Webster 2002). Most of these chemicals influence the production of gibberellins, which are group of natural plant growth regulators vital for normal growth. When the synthesis of gibberellic acid is inhibited then internode extension can be very limited. The application of these inhibitors, therefore, produces a tree that is reduced in size because internode extension is limited. It has always been important to ensure that chemicals like 'Cultar' are applied at the appropriate dose, as growth could be entirely stopped at too high a concentration. These chemicals can also persist for long periods of time in both the soil and the tree. More recently, another gibberellin inhibitor, prohexadione-Ca ('Apogee') has been introduced and is much less environmentally persistent than 'Cultar'.

Physical manipulation of root : shoot ratio

There is an innate balance between the dry matter partitioned to the shoot and its root. This balance defines and co-ordinates the relationships between processes taking place within the shoot and root. For example, to maintain a sufficient supply of water to the transpiring shoot requires a fixed amount of root matter for water uptake. Events that disturb this balance, such as removing part of the shoot, perturb this relationship which subsequent growth will attempt to re-establish. This is why shoot or root pruning only has a temporary influence and why there is a constant requirement for further pruning. Removing part of the shoot may also reduce the number of floral buds.

Regulation of water supply through the use of techniques such as partial root drying (PRD) and regulated deficit irrigation (RDI) can provide effective means of reducing water use and enhancing plant water efficiency as well as a way to influence vegetative and reproductive growth (Chalmers *et al.* 1981and 1986).

Another tool for reducing shoot growth of temperate fruit trees involves control of root growth. Pruning of tree roots has been used for centuries to reduce excessive shoot growth and is still used for this purpose occasionally (Geisler and Ferree 1984, Ferree 1989 and 1992, Ferree *et al* 1992, McArtney and Belton 1992; Poni *et al. 1*992). A more recent and novel approach involves the restriction of root growth and development by growing trees within semi-permeable membranes buried in the soil (Myers 1992; Costa *et al.* 1992; Erez *et al.* 1992; Atkinson *et al.* 2000b; Webster *et al.* 2000; Webster *et al.* 2003). Experimental studies have shown, with apple, that through root-restriction (by planting trees within root confining, but water permeable fabric membranes) vigorous rootstocks can be made to behave in a way similar to those of dwarfing rootstocks. The observed reductions in shoot growth due to restricted root growth were achieved in the absence of changes in plant water relations, suggesting that water shortage was not the growth inhibitor (Atkinson *et al.* 2000b).

Success with other crops

Rootstocks exist for many other temperate fruit crops including for *Pyrus* (pear), *Prunus* (apricots, cherry, peach, and plum), including several nut crops: *Prunus* (almond), *Juglans* (walnut), *Corylus* (hazelnut), *Carya* (pecan), *Pistacia* (pistachio), as well as *Vitis* (grape). Other crops where rootstocks have shown promise include tea where enhancement of yield has been evident (Bore 1997). In Table 1, a genera list has been included from work of Tubbs (1967) to demonstrate the broad nature of proven and

probable rootstock/scion interactions recorded with woody perennials. The number is now likely to be much greater.

Potential for using dwarfing-type planting systems in cocoa

The level of sophistication of cocoa production varies markedly with respect to the region of its cultivation in the world. In some areas the fairly simplistic approach to its cultivation and the selection of material, for propagation, focuses on vegetative vigour. Selection based on vegetative vigour alone is likely to yield trees with potential high yields, at least, on a per tree basis. However, to achieve and sustain high yields requires intensive management (nutrition, pest and disease control and maintenance of adequate light interception, *etc.*). Under such circumstances control of tree size in cocoa is likely to become an issue as trees age and achieve full leaf canopies. If planting densities are not appropriate for the growth and cropping potential of a given cultivar then light interception becomes crop limiting. In the absence of the type of tree management seen with temperate perennial crops, such as apple, growth potential and cropping will likely decline, due to both within and between tree competition for light *etc.*

To sustain cropping potential under any growing system requires an empirical understanding of the most appropriate planting density. Determining the most appropriate density is based on how quickly the tree fills its allotted canopy space. This is related to density at planting and vigour of growth. For cocoa, the selection of cropping performance based on high vigour, because trees with high vigour have a greater potential to crop, is only sustainable providing planting density is 'correct' and has taken into account factors such as vigour, climatic conditions, level of tree management inputs, nutrition *etc.* In low intensity production of cocoa, it may be the case that selection for vigour is only useful providing a low density planting system is used. Under low density planting systems yields per unit land area would initially be potentially low, but increase until canopy closure.

The advantage of a planting system in which vegetative growth is restricted is that it enables high density planting to be used and allows cropping potential to be induced early in the plant's life. If excessive vegetative growth can be restricted then the management input can be reduced and yield efficiency of high-density plantings can be greater than that from more vigorous trees.

Conclusions

- Scion dwarfing, in apple, using rootstocks has been developed over 100's of years.
- Rootstocks were originally used and have subsequently been selected, so that valued scion (for fruit quality) clones could be propagated.
- The benefits of using dwarfing rootstocks in temperate tree crops are numerous and include improved precocity, enhanced yields and reduced orchard management costs.
- Rootstocks increase harvest index and dwarfing increases yield efficiency even more.
- No complete mechanistic understanding of how rootstocks dwarf scions exists.
- Without the benefits of reduced tree size the temperate fruit orchard would not be economically viable.
- Alternative methods to control vegetative growth exist and include chemical and physical techniques.

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Table 1. Tentative list of genera in which proven or probable rootstock/scion influence has been noted in woody perennials (taken from Tubbs 1967)

	GYMNOSPERMAE-CONIFERAE	
	Paceae	*Abies*
		Picea
		Pinus
		Larix
	Juniperae	*Juniperus*
	ANGIOSPERMAE-DICOTYLEDONEAE	
Piperales	*Piperaceae*	*Piper*
Salicales	*Salicaceae*	*Populus*
Juglandales	*Juglandaceae*	*Juglans*
Fagales	*Betulaceae*	*Betula*
	Fagaceae	*Nothofagus*
Urticales	*Ulmaceae*	*Celtis*
		Zelkova
Ranales	*Magnoliaceae*	*Michelia*
		Magnolia
	Calycanthaceae	*Chimonanthus*
	Anonaceae	*Anona*
	Lauraceae	*Persea*
Rosales	*Saxifragaceae*	*Ribes*
	Rosaceae	*Cotoneaster*
		Malus
		Crataegus
		Sorbus
		Aronia
		Eriobotrya
		Amelanchier
		Chaenomeles
		Cydonia
		Pyrus
		Rosa
		Prunus
	Leguminosae	*Cytisus*
		Robinia
		Cladrastis
		Colutea
		Halimodendron
		Caragana
		Calophaca
Geraniales	*Rutaceae*	*Citrus*
	Meliaceae	*Lansium*
	Euphorbiaceae	*Hevea*
Sapindales	*Anacardiaceae*	*Mangifera*
	Celastraceae	*Euonymus*
	Aceraceae	*Acer*
Rhamnales	*Vitaceae*	*Vitis*
	Malvaceae	*Hibiscus*
	Sterculiaceae	*Theobroma*
Parietales	*Theaceae*	*Camellia*
Myrtiflorae	*Thymelaeaceae*	*Daphne*
	Elaeagnaceae	*Elaeagnus*
Ebenales	*Sapotaceae*	*Sapota*
Contortae	*Oleaceae*	*Syringa*
		Ligustrum
		Olea
		Chionanthus
Tubiflorae	*Solanaceae*	*Solanum*
Rubiales	*Rubiaceae*	*Coffea*
	Caprifoliaceae	*Sambucus*

A Cocoa Growth Mutant with a Dwarfing Effect as Rootstock

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Abstract

A mutant, MJ 12-226, with abnormal growth characteristics was identified at the Cocoa and Coconut Institute (CCI, formerly CCRI) of Papua New Guinea (PNG). The mutant, found among progenies of the cross SCA 12 x NA 149, was characterised by small and narrow leaves, a small root system and strong branching habit. When used as a rootstock, the mutant had a significant dwarfing effect on scion growth of three clones in the nursery. The dwarfing effect has persisted in the field so far one year after planting. Genetic studies showed that the growth mutant was probably heterozygous DM_1dm_1 and the mutant phenotype was determined by the dominant allele DM_1. However, mutant segregants were obtained in a 1:1 ratio only when the mutant clone was used as the female. When the mutant was used as male, very few mutant segregants were obtained. This suggested a possible interaction between nuclear and cytoplasmic genes, or unequal germination or pollen tube growth rate of the DM_1 and dm_1 pollen grains. A homozygous DM_1/DM_1 plant has not been identified yet. The strong branching habit of the mutant suggested weak apical dominance resulting from a change in the quantity or balance of growth hormones. This was supported by accelerated bud sprouting on the mutant rootstock compared with normal rootstock.

Introduction

Improved harvest index is a major target of breeding programmes. In fruit trees, an improved harvest index can be achieved by the use of dwarfing rootstocks. This is exemplified in the well known case of apples. Dwarfing rootstocks can reduce tree size to improve harvest index and allow the planting of more trees per unit area leading to increased production. Small trees require less pruning and are easier to harvest and thus their cultivation incurs lower management costs.

In cocoa, rootstocks with known attributes have not been developed. Close tree spacing of cocoa in the field has therefore failed: trees have grown too large and too rapidly to manage economically (Purdy and Eskes 2002). Preliminary studies at the Cocoa and Coconut Institute (CCI) in Papua New Guinea (PNG), have shown that when cocoa hybrids with differing potential vigour were used as rootstocks, there was no apparent effect on the growth and yield of the scions (budded clones), nor an interaction effect of rootstocks x scions (Efron and Epaina, unpublished).

More recently, a mutant with abnormal growth characteristics was identified at CCI. Several studies were conducted with the mutant to determine its importance as a rootstock and to understand its genetic control (Efron *et al.* 2002, 2003a, 2003b). The following paper summarises the results obtained so far on the characterisation of the mutant, its dwarfing effect on the budded scions and the likely genetic control of the mutant.

Materials and Methods

The cocoa growth mutant was identified as a single progeny tree of the cross SCA 12 x NA 149. A clone, MJ 12-226, was established from it by orthotropic budding on normal rootstock. Characterisation of the mutant and its effect as rootstock were mostly done on mutant and normal segregants obtained from the same open-pollinated pods harvested from the clone MJ 12-226.

Genetic studies included open-pollinated pods of MJ 12-226 and controlled hand-pollination of MJ 12-226 crossed as female with the clones OTC 1, Matina 1-9, KA2-101 and EET 308 as males. Reciprocal crosses were made with KA2-101, EET 308 and KEE 42 as females crossed with pollen from the mutant clone MJ 12-226.

The clones 21-4-8, 17-3/1 and 37-13/1 were used as scions to study the effect of the mutant as a dwarfing rootstock. Plagiotropic buds of the clones 21-4-8, 33-15/1 and the mutant clone MJ12-226 as well as orthotropic buds of the clone 33-15/1 were used in one experiment as scions to study the effect of the mutant on sprouting rate. A second experiment included orthotropic and plagiotropic buds of the clones 17-3/1, 33-15/1 and 37-13/1 as scions.

Grafting was done by patch budding on 2-3 month-old seedlings when mutant and normal segregants were clearly distinguishable. Budding tapes were removed two weeks after budding and sprouting was assessed thereafter at two day intervals.

Height, leaf width and length, dry matter and stem diameter were the variables used to measure vegetative growth.

Results

The Clone MJ12-226

The clone MJ12-226 continued to show the mutant characteristics of short growth with small and narrow leaves and profuse branching after planting in the field. The trees continued to be shorter than other grafted clones from the same cross for the first two years. Later, the differences in height with the neighbouring clones were less pronounced due to pruning. An average of 19.3 pods/tree was harvested from January to September 2001, which was similar to the two neighbouring clones (17.8 and 24.5 pods/tree). Further comparison was not possible because the pods from MJ12-226 were used for the various studies and for controlled pollinations. The pods were also of normal size (412 g) with 37.2 seeds/pod. The seeds were small (less than 1 g) which is typical of the cross SCA 12 x NA 149. The clone MJ12-226 is probably self-incompatible. More than 100 self-pollinations were attempted, but all failed.

Growth characteristics of mutant and normal segregants

The mutant and normal segregants were obtained from open-pollinated pods of the clone MJ12-226. Initially, after germination, all the seedlings looked the same. The mutant and normal phenotypes were clearly distinguishable only after about two months. The mutant plants were shorter with smaller leaves and less developed root systems (Figure 1). Most of the mutant seedlings produced multiple stems that developed into multi-stemmed short and compact trees (Figure 2). The first fan branches after jorquetting also had a strong branching habit (Figure 3).

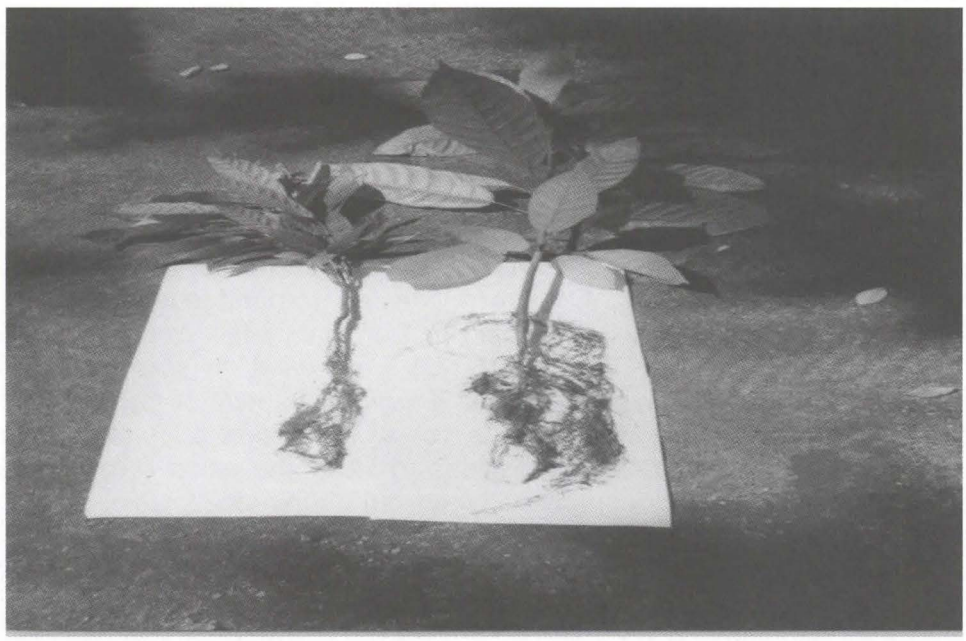

Figure 1. Mutant (left) and normal (right) seedlings derived from seeds of the same open-pollinated pods

Figure 2. A mutant tree with multiple orthotropic stems (front) and a normal tree derived from the same pod and planted at the same time (back)

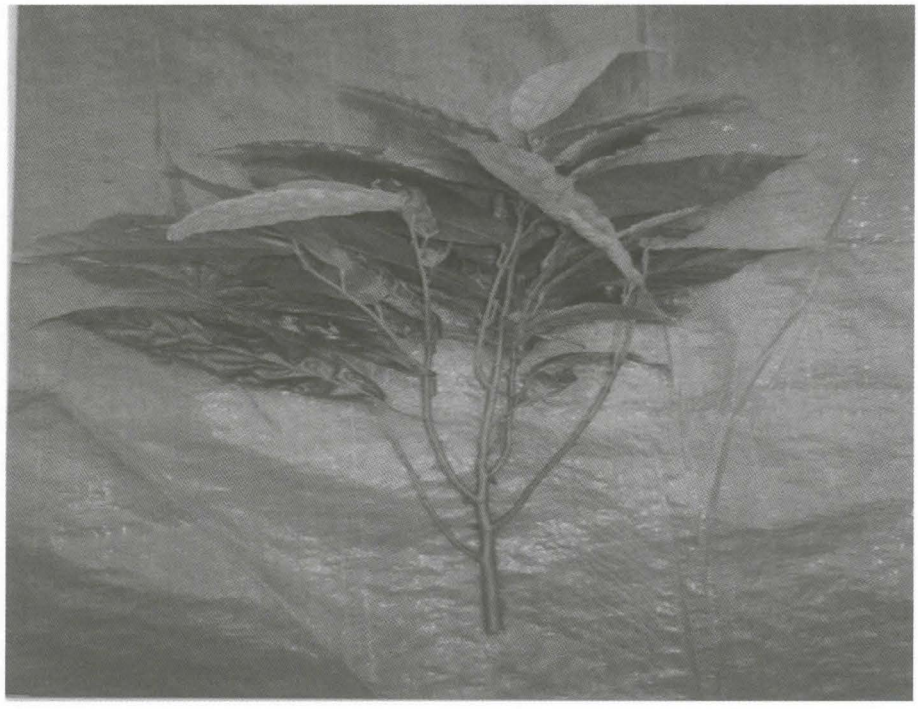

Figure 3. The fan branch growing from the jorquette point of mutant tree. (Note the strong branching habit.)

Measurements of seedling height, leaf length and leaf width of the mutant and normal segregants four months after planting showed clear differences between the two phenotypes (Table 1). The two groups of plants were completely separated, with no overlapping of measurements of individual plants as was reflected by the range obtained. Leaf width was the most affected variable. The difference between the mutant and normal segregants measured as dry weight grew bigger with time, particularly between five and six months after planting (Table 2). The biggest difference between the two groups was obtained in the dry weight of the leaves.

Genetic control

All the crosses with the mutant clones as female showed a significant fit to a 1:1 ratio between the normal and mutant phenotypes (Figure 4, Table 3). A similar ratio was obtained from open-pollinated pods. However, when the mutant clone MJ12-226 was used as male parent, all except 10 of the 585 seedlings tested showed a normal phenotype.

Reciprocal differences were also observed in the relative growth rate of the normal phenotypes (Figure 5). The average height of 10 normal seedlings from the cross EET 308 x MJ12-226 was significantly higher by 24.1% than the average height of 10 normal seedlings from the reciprocal cross MJ12-226 x EET 308. This

Table 1. Average[1] plant height, leaf length and leaf width of three month old normal and mutant phenotypes derived from open pollinated pods from the clone MJ 12-226

Variable	Phenotype		Mutant/normal (%)
	Normal	Mutant	
Seedling height (cm)	48.2 (43-54)	32.9 (24-37)	68.3
Leaf length (cm)	25.8 (23-29)	16.0 (11-19)	62.0
Leaf width (cm)	9.5 (9-10)	4.5 (3-5)	47.4

1) Average of 10 seedlings. The range is given in brackets

Table 2. Average[1] dry weight (g/plant) of plant components of the mutant and normal segregant seedlings measured at 5, 6 and 7 months after sowing

Plant component	Age of seedlings (month)								
	5			6			7		
	N	M	M/N (%)	N	M	M/N (%)	N	M	M/N (%)
Roots	0.86	0.76*	88.4	2.18	1.25*	57.3	3.13	2.09*	66.8
Stem	0.55	0.56	101.8	1.62	1.03*	63.6	3.27	1.62*	49.5
Leaves	1.68	0.81*	48.2	3.92	1.52*	38.8	7.00	2.60*	37.1
Whole Plant	3.09	2.12*	68.6	7.71	3.79*	49.2	13.40	6.31*	47.1

1 = average of four reps, 10 seedlings/rep.
N = Normal rootstock M = Mutant Rootstock
* = Significantly different from the normal for each plant component at the 5% level

was confirmed later in a larger experiment using nine normal segregants obtained from six different pods for each of the reciprocal crosses, replicated six times whereby the seedlings from each pod were used as a separate replication. After three months, the seedlings were planted in the field. Plant height and stem diameter were measured 3, 6 and 9 months after sowing. The difference in plant height was maintained (about 20-25%) with time (Table 4). However, the difference in stem diameter, whicht was 26% at the age of three months, was reduced to only 10% at the age of nine months.

Bud sprouting on mutant rootstocks

Sprouting started at day 16, two days after removal of the budding tape. Plagiotropic buds started to sprout earlier and at a faster rate than orthotropic buds (Figure 6a). Among the clones, 33-15/1 started to sprout later and at a slower rate than 21-4-8 on both the normal (Figure 6b) and the mutant (Figure 6c) rootstocks. However, the

Table 3. Number of normal and mutant segregants and goodness of fit to a 1:1 ratio in reciprocal crosses between the cacao growth mutant MJ12-226 and several normal clones

Cross	Number of seedlings		Total	X^2	P
	Normal	Mutant			
MJ12-226 O.P	416	392	808	0.65	0.25 – 0.50
MJ12-226 x OTC-1	136	154	290	0.99	0.50 – 0.75
MJ12-226 x Matina 1-9	114	98	212	1.06	0.10 – 0.25
MJ12-226 x KA2-101	117	135	250	1.17	0.75 – 0.90
MJ12-226 x EET 308	363	347	710	0.32	0.75 – 0.90
Total	**1146**	**1126**	**2272**	**0.16**	**0.75 – 0.90**
KEE 42 x MJ12-226	212	0	212		
KA2-101 x MJ12-226	102	4	106		
EET 308 x MJ12-226	261	6	267		
Total	**575**	**10**	**585**		

Table 4. Average[1] plant height and stem diameter of normal segregants from the reciprocal cross, EET 308 x MJ 12-226 and MJ 12-226 x EET 308 at three, six and nine months after sowing

Parameter	Cross	Age of seedling (months)		
		3	6	9
Plant height (cm)	EET 308 X MJ 12-226 (M)	43.7**	58.2**	70.3**
	MJ 12-226 X EET 308 (F)	32.9	46.9	54.0
	F/M (%)	**75.3**	**80.5**	**76.8**
Stem diameter (mm)	EET 308 X MJ 12-226 (M)	4.1**	7.6*	11.2
	MJ 12-226 X EET 308 (F)	3.0	6.3	10.1
	F/M (%)	**74.0**	**82.8**	**90.2**

1 = Average of 6 replicates, 9 plants/replicate.
*, ** = Significantly and highly significantly different from the reciprocal cross at the 5% and 1% level, respectively (Newman-Keuls's test)

most noticeable effect on the rate of sprouting was obtained with the mutant genotype either as a scion or, in particular, as a rootstock. The fastest sprouting rate was obtained with plagiotropic buds of the mutant budded onto the mutant rootstock, whereby at day 20 more than 90% of the buds had sprouted (Figure 6e).

Table 5. Progressive sprouting (%) of plagiotropic and orthotropic buds of the clones 17-3/1, 33-15/1 and 37-13/1 budded on normal and mutant rootstocks

Clone	Bud type	Root stock	Strike rate (%)	Percent sprouting days after budding						
				16	20	24	28	32	36	40
17-3/1	P	N	100	27	45	65	83	90	97	97
		M	100	47	76	84	91	97	98	98
		D	-	20	31	19	8	7	1	1
	O	N	100	7	12	20	40	60	80	85
		M	100	23	40	57	67	68	80	85
		D		17	28	37	27	0	0	0
33-15/1	P	N	100	8	30	53	82	97	97	97
		M	98	38	68	75	82	88	92	92
		D		30	38	22	0	-9	-5	-5
	O	N	93	0	5	13	28	61	66	72
		M	92	7	20	48	60	65	82	83
		D		7	15	35	32	4	26	11
37-13/1	P	N	100	10	20	47	62	85	85	85
		M	100	22	58	72	77	93	95	95
		D		12	38	25	15	8	10	10
	O	N	98	3	8	18	30	65	67	72
		M	77	8	13	30	52	63	67	68
		D		5	5	12	22	-2	0	-4

P = Plagiotropic, O = Orthotropic, N = Normal rootstock, M = Mutant rootstock,
D = the difference between the mutant and normal rootstock

Table 6. The effect of normal and dwarf mutant rootstocks on height, leaf length and leaf width of three budded clones[1]

Variable	Clone								
	21-4-8			17-3/1			37-13/1		
	N	M	M/N (%)	N	M	M/N (%)	N	M	M/N (%)
Height (cm)	24.8	16.2**	65.3	31.4	17.8*	56.7	29.4	22.2**	72.1
Leaf length (cm)	27.0	23.0*	85.2	25.2	19.8	78.6	26.2	21.0*	80.1
Leaf width (cm)	8.6	6.5**	75.6	9.4	7.5*	78.1	8.3	8.0	97.1

1 = Average of four replicates, 10 plants/replicate
N – Normal rootstock M – Mutant rootstock
*, ** - Significantly and highly significantly different from the normal at the 1% and 5% level, respectively

The accelerated sprouting on the mutant rootstock was obvious in both plagiotropic budding of the clone 21-4-8 up to day 24 (Figure 6f) and orthotropic budding of the clone 33-15/1 up to day 32 (Figure 6d). Very similar results were obtained in the second experiment (Table 5).

Table 7. Stem diameter, plant height and canopy width of three clones budded on mutant and normal rootstocks one year after field planting

Scion	Rootstock	Stem diameter (mm)	Plant height (cm)	Canopy width (cm)
21-4-8	N	40.7	145.6	124.9
	M	31.5 (77.4)	111.9 (76.8)	70.3 (56.3)
17-3/1	N	44.5	144.8	125.9
	M	34.9 (78.4)	121.0 (83.6)	94.6 (75.1)
37-13/1	N	45.2	147.3	137.4
	M	33.9 (75.0)	115.3 (78.3)	99.4 (72.3)
Average	N	43.5 **	145.9 **	129.4 **
	M	33.4 (76.9)	116.1 (79.6)	88.1 (68.1)

Numbers in brackets refer to M/N x 100
** Significantly higher at the 1% level

The dwarfing effect of the mutant as rootstock

The dwarfing effect of the mutant rootstock on scion growth was clearly visible in the nursery when the same clone was budded onto mutant and normal segregants as rootstocks (Figure 7). The major effect was observed on seedling height (Table 6). Leaf length and width were also reduced but to a lesser extant.

The difference in scion growth was maintained one year after planting (Table 7). Stem diameter, plant height and canopy width of the three clones, 21-4-8, 17-3/1 and 37-13/1, were measured. There were no significant differences between clones budded on the same rootstock. However, there were highly significant differences between the two rootstocks. These differences were similar for the three clones and the three variables.

Discussion

The growth characteristics of the mutant and, in particular, its smaller root system suggested that it can possibly be used as a dwarfing rootstock. The results obtained with several clones in the nursery confirmed this assumption whereby the seedlings budded on the mutant rootstock were significantly shorter than the same clones budded on normal segregants. These differences persisted in the field one year after planting. However, the field observations are very preliminary. Additional information is required over time, particularly regarding the effect of dwarfing on yield potential and the optimal planting density. A larger scale, new experiment was recently planted in the field.

Moreover, the mutant genotype dramatically changes the shape and size of the cocoa tree. With appropriate breeding efforts, cocoa hybrids and clones with a dwarf mutant phenotype can be developed. The resulting cocoa field will look completely different from the cocoa plantations that we are used to seeing today. Higher densities of planting would be possible. Only time will tell if the yield potential can also be improved.

A 1:1 segregation ratio is usually obtained in test crosses between heterozygous and homozygous genotypes. The uniformity of the 1:1 segregation ratio between normal and mutants obtained in several crosses when the mutant

clone was used as female, including open-pollinated pods from the mutant clones MJ12-226, suggested that the mutant phenotype was determined by a dominant allele (DM_1) and the clone MJ12-226 is heterozygous DM_1dm_1. The genotype of the normal clone is homozygous recessive dm_1dm_1 and the progenies segregate in a 1:1 ratio of DM1dm1 (mutant) to dm_1dm_1 (normal). The data obtained fit this model well.

An interaction between nuclear and cytoplasmic genes may explain the reciprocal differences. Accordingly, the cytoplasm of the mutant MJ12-226 (n) is different from the normal cytoplasm (N) and the DM_1 allele can express the mutant phenotype only in the mutant (n) cytoplasm as follows:

Cytoplasm	Genotype	Phenotype	Seedling height
N	DM_1dm_1	normal	normal
N	dm_1dm_1	normal	normal
n	DM_1dm_1	mutant	dwarf
n	dm_1dm_1	normal	intermediate

The observed difference in seedling height of normal segregants from the reciprocal crosses supports the hypothesis of the existence of different types of cytoplasm, and suggests that it is possibly due to mitochondrial genes that are involved in the mechanism of energy production. Transmission of male mitochondria (Motamayor, personal communication.) can explain the appearance of very few mutant phenotypes when MJ12-226 was used as male parent. Similar examples of interaction between nuclear and cytoplasmic genes are well known *e.g.* male sterility in maize and sorghum.

The reciprocal differences may also be explained by an alternative hypothesis. When the mutant clone (DM_1dm_1) was used as male parent, there were two types of pollen grain, DM_1 and dm_1. It is possible that the DM_1 pollen grains were either not viable or their pollen tubes grew more slowly than those of the dm_1 pollen grains. Therefore, most of the ovules were pollinated by dm_1 pollen grains. This hypothesis explains the very low frequency of mutant phenotypes obtained when the mutant clone was used as a male parent. However, it does not explain the reciprocal differences in seedling growth. The reciprocal differences in seedling growth may possibly also be attributed to the relatively large seed size of EET 308 compared to that of MJ12-226. Additional studies are required to differentiate between the two models proposed to explain the reciprocal differences.

Currently, only 50% of the seedlings show the mutant phenotype. They can only be identified as mutants at about two months after planting. If the dwarfing effect of the mutant could be of value to in obtaining smaller trees with improved harvest index, it will be necessary to find a way to increase the proportion of mutant segregants.

Therefore, it is important to continue with the genetic studies.

Figure 4. Segregating mutant and normal seedlings from the cross MJ12-226 x KA2-101 (front). The seedlings at the back are all normal from the reciprocal cross KA2-101 x MJ12-226

Figure 5. Normal seedlings from the cross MJ12-226 x EET 308 (left) and the reciprocal cross EET 308 x MJ12-226 (right)

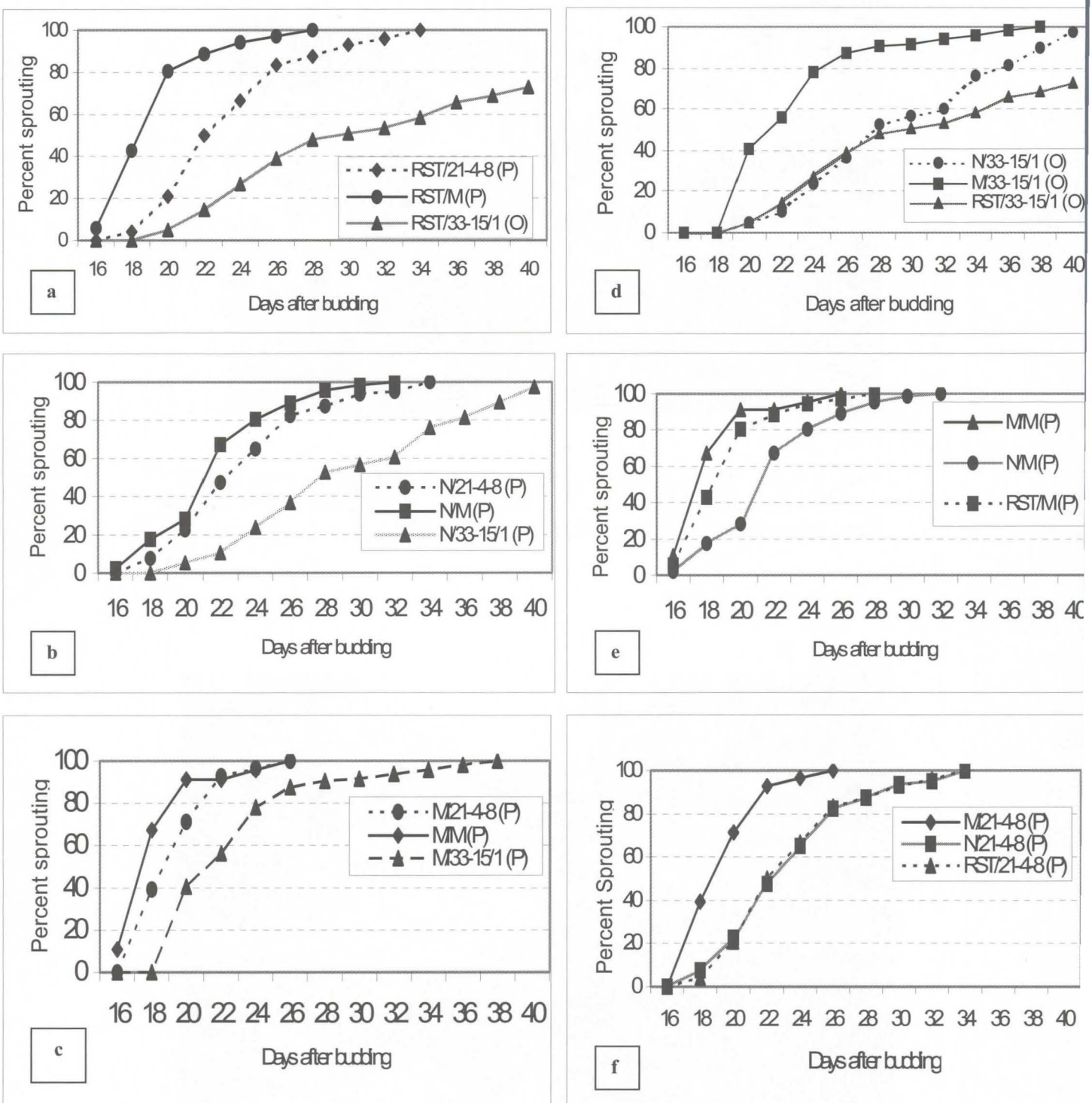

Figure 6. Progressive sprouting rate (%) of plagiotropic and orthotropic buds in various rootstock and scion combinations where P = Plagiotropic, O = Orthotropic, N = normal segregants, M = mutant segregants, RST = common rootstock

Figure 7. Mutant (far left) and normal (far right) seedlings, and the orthotropic budded clone on mutant (middle left) and normal (middle right) seedlings as rootstocks

In particular, it is important to develop a homozygous DM_1DM_1 genotype either by selfing of the mutant genotypes or crossing two different mutant segregants. If viable and productive, all its progenies should be of the dwarf type. The mutant clone MJ12-226 itself is probably self-incompatible.

The reciprocal differences in the growth rate of the seedlings and the possible effect of the cytoplasm are of particular interest. If they are verified and the differences in growth rate persist with time, the use of the mutant cytoplasm may provide a way to reduce tree vigour of both hybrids and clones, which are unrelated to the rootstock.

Cocoa clones are mostly propagated by budding or cuttings from plagiotropic branches. The grafted plants do not grow straight and formation pruning is required to direct the plants to grow upright in a balanced way. Budding can also be done with orthotropic buds. The budded trees grow like seedlings with a single upright growing stem and formation pruning is not required. If orthotropic budding can be done on a commercial scale, recently released hybrid clones would be more easily accepted by cocoa growers, particularly in the small scale sector. However, one of the major problems with orthotropic budding is that orthotropic buds tend to be dormant and therefore sprouting takes longer and is not uniform (Efron *et al.* 2000).

The peculiar growth habit of multiple orthotropic stems and the strong branching habit of fan branches in the mutant suggested weak apical dominance. As apical dominance and bud dormancy are affected by plant hormones, probably Indole Acetic Acid (IAA), it was hypothesized that buds grafted onto the mutant rootstock would sprout faster and more uniformly than the same buds grafted onto

normal rootstock. This hypothesis was confirmed by the experimental results obtained with both plagiotropic and orthotropic buds.
Assuming that bud dormancy and sprouting are affected by hormones, a comparative study between the normal and the mutant root stocks can provide an insight into the type and concentration of hormone(s) that can break dormancy and induce faster bud sprouting. Based on this information, technology to induce faster and more uniform sprouting of orthotropic buds by artificial application of plant hormones of the right type and concentration may be developed.

Acknowledgements

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Investigation into the Use Of the Crinkle Leaf Mutant as a Low Vigour Rootstock for High Density Planting in Cocoa

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Abstract

Work was carried out at CRIG to investigate the possible use of the Crinkle Leaf Mutant (CLM) of cocoa, which is small in structure and slow in growth, as a low vigour rootstock to produce slow and low vigour cocoa clones that could be used for high-density cocoa plantings. Physiological and genetical aspects were studied as well as early field performance. The physiological studies showed that the biomass production and distribution, between roots and shoots, as well as the levels of total free sugars were markedly lower in the CLM than in normal seedlings segregating in the same progenies. This was more clearly discernible in the roots. Varietal differences in graft-take were observed with Amelonado being a poor scion on the mutant rootstock. Initial results from field growth studies suggest that the CLM rootstock may reduce shoot growth. In crosses involving two CLM as females, T2 and T100, crossed with Amelonado, NA 32 and IMC 67 and in selfings, significant differences were observed between crosses in terms of production of CLM segregants. Selfed T2 produced a significantly higher proportion of CLM than any of the other progenies. The crinkle: normal (C:N) ratio fitted a 2:1 ratio indicating dominance for the trait. The indications are that the CLM has potential as a low vigour rootstock and can be exploited for high density planting.

Introduction

High density planting in cocoa, as in other plants, will theoretically increase yield. However, in cocoa, the yield increases are not easily sustained. Under such high density planting, cocoa grows rapidly and the trees become too large in size to manage, resulting in poor economic gains. However, reduction in both tree vigour and size could reduce the intense competition associated with close planting in cocoa and therefore enhance economic gains. Some of the options that could be adopted to obtain cocoa trees with less vigour and a small stature that is more suited to close density planting are breeding and selection of such clones and the use of rootstocks that control scion growth and size (Purdy and Eskes 2002). The latter option is being considered in the investigation reported here.

Among the Trinidad introductions made to Ghana by Posnette (1948), one tree was discovered which had crinkle leaves and was named "Crinkle Leaf Mutant" (CLM), coded as T2. The tree, apart from the crinkle leaves, also has short internodes, is small in stature and slow in growth. The pod and bean size of the CLM mutant are also reduced. Seeds from any open-pollinated pod from the CLM mutant will produce seedlings with either normal leaves or crinkle leaves, roughly in a 2:1 ratio (Adu-Ampomah *et al.* 1998). Whilst the crinkle leaf mutant is slow in growth and small in size, the normal siblings from the same pod are vigorous and produce larger plants.

Another tree at Tafo (coded T100), which is a progeny from a cross between T2 and NA 32 (Lockwood and Gyamfi 1979), has similar characteristics to T2 except that T2 has pigmented new flushes whilst T100 does not. In this study, we have

investigated some physiological attributes of CLM plants, the possibility of controlling crosses to produce high proportions of mutant seedlings and, to a limited extent, the effects of the low vigour mutant as a rootstock on the growth and size of scions growing on them.

Materials and Methods

Physiological studies

The following physiological traits were investigated: biomass production and distribution, total free sugars and leaf chlorophyll six months after planting, number of leaves per plant, stem diameter and stem height.

Genetic studies

The following crosses were carried out:

1.T2 x Amelonado (AMEL)
2.T2 x NA 32
3.T2 x PA 7
4.T2 x IMC 67
5.T2 x T100
6. T100 x AMEL
7. T100 x NA 32
8. T100 x PA 7
9. T100 x IMC 67
10. T100 x T2
11.T2 selfed
12. T100 selfed
13. PA 7 x P 30 (Amelonado clone)

Four trees of each female mutant clone type were used with each tree representing a replicate. This was to offset the possible nutrient variation effects in the soil. Lack of some micronutrients is known to induce crinkle leaves in some plants. Twenty beans of each cross were selected at random and planted in aluminium trays filled with sterile soil. This was replicated for each cross three times. The number of CLM plants and normal seedlings were noted at the end of 12 weeks. The control cross was PA 7 x P 30.

Field studies

The following rootstocks were raised:

(1) crinkle leaf seedlings
(2) normal seedlings from CLM pods
(3) open-pollinated seedlings from Amelonado
(4) hybrid seedlings (T85/799 x AMEL).

Budwood from NA 33 (slow growing), P 30 (Amelonado, slow growing), and T79/501 (fast growing) were budded onto fifty each of the above rootstocks and planted in a replicated trial in the field at 8'x 8' (10 plants/plot with five replicates).

Results and Discussion

Physiological studies

Table 1 shows the height per plant, stem diameter, number of leaves per plant and the fresh and dry weights of the various parts of the seedlings. These data clearly show differences in biomass production and distribution with the crinkle leaf mutant seedlings being smaller in size and slower in growth than the normal cocoa seedlings. These differences were most obvious in the measurements made of the roots.

Table 1. Growth, biomass production and distribution in crinkle leaf and normal cocoa seedlings at 6-months old

Progeny	Height (cm)	Stem diame-ter	No leaf/ plant	Fresh weight (g)			Dry weight (g)			Total DW(g)
				Root	Stem	Leaf	Root	Stem	Leaf	
T85/799 X Amel	54.4	0.51	13.8	4.8	5.6	9.0	1.46	1.64	3.23	6.32
T2 (crinkle)	25.0	0.44	12.0	0.9	3.2	3.0	0.28	0.71	1.06	2.05
T2 (normal)	48.6	0.51	11.8	4.7	5.7	0.1	1.20	1.68	2.74	5.62
T100 (crinkle)	23.5	0.44	13.1	1.0	3.1	3.2	0.30	0.70	1.10	2.10
T100 (normal)	55.0	0.50	12.4	4.7	5.7	5.7	1.24	1.70	2.70	5.64

Table 2. Comparison of total free sugar and leaf chlorophyll of CLM and normal seedlings

Progeny	Total free sugar (mg/g.dw)		Leaf chlorophyll content (mg/l)
	Root	Stem	
T85/799 x Amel (normal)	253.3	200.9	1.03
T2 (crinkle)	151.2	222.3	0.87
T2 (normal)	181.4	210.9	1.30

Table 2 shows the total free sugars in the roots and stem and the chlorophyll content of the leaves. The total free sugars in the crinkle leaf mutants were markedly lower in the roots than in the roots of the normal seedlings, but in the stem they are not markedly different from each other. Even though the leaf chlorophyll content of the CLM was lower, this was not very different from that in the normal seedlings. The relatively lower levels of free sugars in the roots of the CLM might account for the reduced growth in the upper shoots (scions).

Grafting success

The influence of the CLM rootstock on graft take was determined by comparing the survival of nine scions on CLM and normal rootstocks after 12 months (Table 3). The results suggest that there could be varietal differences with regards to graft take, with Amelonado being a very poor scion on the CLM rootstocks.

Field performance

The initial data for scion growth on the CLM rootstocks in the field are still scanty and therefore not presented here. However, there is a general reduction in shoot vigour with scions grafted onto CLM rootstocks in relation to normal hybrid rootstocks.

Table 3. Rootstock (CLM T2 and T100 types) influence on graft take (after 12 months)

Rootstock x scion combination	Graft take (%)
T2 x NA 33	75.0
T2 x T79/501	68.3
T2 x Amelonado	0.0
T100 x NA 33	100.0
T100 x T79/501	75.0
T100 x Amel	0.0
Amel x NA 33	50.0
Amelonado x Amelonado	100.0
Amel x T79/501	30.0
Mean	55.4
S.D.	36.1

Genetic analysis Table 4 presents the percentage of crinkle leaf mutants per cross. As expected, no significant differences were observed between the female trees (different trees within clones) and no interactions between crosses and female trees were observed. However, significant differences

Table 4. Averages and differences (according to least square means) for the percentage of segregating seedlings with the CLM trait in 13 different crosses (total number of 240 seedlings per cross)

Crosses	Mean (% CLM seedlings)	Significance of differences between crosses (based on T test of mean squares)	Total CLM seedlings in relation to normal seedlings
A = T2 x PA 7	65.0	B	1.86 : 1
B = T2 x PA 30	68.3	B	2.16 : 1
C = T2 x IMC 67	65.8	B	1.93 : 1
D = T2 x NA 32	70.4	B	2.38 : 1
E = T2 x T100	70.0	B	2.33 : 1
F = T2 selfed	80.4	A	4.0 : 1
G = T100 x PA 7	63.3	B	1.73 : 1
H = T100 x P 30	67.5	B	2.08 : 1
I = T100 x IMC 67	64.6	B	1.82 : 1
J = T100 x NA 32	60.2	B	1.53 : 1
K = T100 x T2	67.5	B	1.86 : 1
L = T 100 selfed	64.6	B	2.16 : 1
M = PA 7 x P 30	0.0	C	1 : 0

were observed between crosses. T2 selfed progeny produced a significantly higher proportion (4:1) of CLM than any of the other crosses, indicating that it is the cross of choice if the mutant is to be exploited on a larger scale as a low vigour rootstock. All the other crosses, aside from the T2 selfed, were not significantly different from each

other. This shows that in the absence of adequate selfings, all the other crosses can also be exploited for provision of CLM for rootstocks.

The C:N ratio of all crosses (except the T2 selfed) fits into a 2:1 ratio (Table 4) indicating some type of dominance in the inheritance of the trait. However, the crosses so effected do not include the use of other females aside from T2 and T100, which are all carrying the CLM trait. A study of crosses involving female parents not carrying the CLM trait or reciprocal crosses (using CLM trees as male parents) could reveal the genetics involved.

Conclusion

The CLM is a slow growing cocoa mutant, which when used as a rootstock, appears to produce "dwarfing" effects on the scion grafted onto it. Crosses made between a variety of clones and the CLM parent resulted in 60-70% CLM seedlings in the progeny and a higher proportion still could be obtained by selfing one of the CLM clones. The indications are that the mutant can be exploited for high density planting.

Acknowledgements

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Impacts of the Micro and Macro Environments on the Genetic Gain by Clonal Selection in Cacao

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Abstract

Preliminary data from six clonal trials were obtained in order to understand the impacts of the micro- and macro-environments on genetic gain obtained through clonal selection. Each trial was planted in 1999 with the same 30 clones at six locations in Bahia State, Brazil, and was assessed for witches´ broom resistance (number of vegetative brooms) and pod production. Clone, location (farms) effects and GxE interactions were highly significant for both traits. It was observed that using 10 trees per clone per location resulted in similar expected genetic gain as that obtained using 20 trees, for both traits studied. Grouping locations by their geographic proximity might not be a good strategy for clone recommendations. Soil factors are more important than geographical distance and climate factors in causing genotype-by-environment interaction for early yield. Selection based on the average of locations results in high gain in individual locations for witches´ broom resistance, but not for pod production.

Introduction

Cacao is planted in various areas of the Tropics, with different types of soils, climates, topographies and husbandry systems, resulting in large macro-environmental variation across plantations. As a result, the best varieties in a given location may not be the best at another. Thus, the indirect genetic gain obtained by selecting in a given location or on the average of all test locations may be reduced by the presence of genotype-by-environment interactions. Most studies in this area, in cacao, have been focused on quantifying the interaction or on identifying more adapted or stable varieties (Mariano *et al.* 1987; Pinto *et al.* 1993; Jolly and de Verteuil 1960; Martin and Lockwood 1979; Cilas *et al.* 1985), none of them in evaluating the impacts of the interaction on the genetic gain. Moreover, besides the detection of interactions and the identification of more adapted or stable varieties, it is of interest to understand the main causes of these interactions in order to either establish recommendations on planting zones for particular varieties or minimise the interaction by homogenising the factor(s) causing the interaction.

Besides the macro-environmental variation, the presence of shade trees, the heterogeneity in size of the cacao trees and the variability in rootstocks within a plantation result in large micro-environmental variation. Contrasting with this is the need for precise and accurate estimates of genetic values of the varieties, in order to rank them adequately to maximise the gain. The number of trees per clone under investigation certainly affects the precision and accuracy of those estimates, and thus the potential gain attainable.

Preliminary data from six cacao clonal trials were used in this study, and the objectives were to estimate: a) the impact of selection based on a single location, or on the average of all locations, on the indirect gain in other locations; b) the effect of different climate, geographic and soil factors on the genotype-by-environment interaction; and c) the effect of a reduced number of trees per clone on the genetic

gain. Eighteen other clonal trials like the six used in this study, involving a total of approximately 150 clones, are being carried out in Brazil and soon will allow us to have better estimates of the factors studied here.

Material and Methods

Clonal trials

Six clonal trials were established on private farms in Bahia, Northeast Brazil, namely: Massaranduba farm (county: Itajuípe), Nossa Senhora da Conceição farm (Ubatã), Santa Úrsula farm (Camacã), Confiança farm (Belmonte), São Francisco farm (Itabuna) and Corcovado farm (Ipiau), as detailed in Table 1. In all trials, 30 cacao clones were planted in a completely randomised single-tree plot design with 20 replicates (plants). The trials were established between October and December 1999 using top grafting of the test clones (scion) on basal chupons of adult trees (rootstock). Details of these trials are described in Lopes *et al.* (2000). Evaluation of the number of vegetative (canopy) brooms and the number of pods and cherelles was initiated one year after grafting, and subsequently carried out at approximately four-monthly intervals. Since the number of measurements was different among the locations, averages per tree and per harvest were used in all analyses.

Table 1. Description of the six locations used in the genotype-by-environment interaction study

Location	Itajuípe	Ubatã	Camacã	Belmonte	Itabuna	Ipiau
Farm	Massaranduba	N.S. da Conceição	Santa Úrsula	Novo Horizonte	São Francisco	Corcovado
Latitude	14°43'S	14°13'S	15°25'S	15°52'S	14°45'S	14°08'S
Longitude	39°21'W	39°31'W	39°30'W	38°53'W	39°13'W	39°44'W
Rainfall [a] (mm/yr)	1500	1140	1430	1685	1700	1106
Temperature[a] (°C)	22.9	23.0	23.0	23.7	23.3	23.2
Rel Humidity[a] (%)	85.0	83.0	84.0	84.2	85.5	83.0
Rootstock	Hybrids Mixture	Local Variety ("Cacau Comum")	Hybrids Mixture	Hybrids Mixture	Hybrids Mixture	Hybrids Mixture
Spacing (m)	3 x 3	Irregular	3 x 3	3 x 3	3 x 3	3 x 3

[a] Annual average for the period 1981-1999

Micro-environmental effects

The effect of the micro-environment on the expected genetic gain was assessed by the impact of the number of trees per clone on the broad-sense heritability and on the ranking of the clones over sites. For this, a sample of 3 to 19 trees was picked at random, with replacement, from the 20 trees per clone available (17 points x 30 samples = 510 samples/clonal trial). For each of the 510 samples/trial, clonal averages and Pearson correlations between those averages and the averages considering all 20 trees were computed. The broad-sense heritability was also computed for each of these samples by:

$$H^2_{(i)} = \frac{\sigma^2_{C(i)}}{\sigma^2_{C(i)} + \sigma^2_{W(i)}}$$

where, $H^2_{(i)}$ is the broad-sense heritability estimated in the ith location; $\sigma^2_{C(i)}$ is the between-clone variance component; and $\sigma^2_{W(i)}$ is the within-clone variance component.

Macro-environmental effects

The effect of the macro-environment on the genetic gain was computed by predicting the direct gain by selecting on the same location where the clones would be planted and indirect gain by selecting on other locations or on the average of all locations. The direct and indirect gains were computed by:

$$G_{j(i)} = H^2_j SD_{j(i)}$$

where, $G_{j(i)}$ is the genetic gain in the jth location, by selection on the ith location; H^2_j is the broad-sense heritability on the jth location; and $SD_{j(i)}$ is the selection differential in the jth location, given by the difference in means of the clones in the jth location, whose superiority was evidenced in the ith location, and the overall mean in the jth location. When *i* is equal to *j*, the direct gain is estimated, otherwise the indirect gain.

The percent gain, relative to the population mean, was estimated as:

$$pG_{j(i)} = [G_{j(i)} / M_j] \times 100$$

where, $pG_{j(i)}$ is the percent genetic gain in the jth location, by selecting on the ith location and M_j is the overall mean of the trial on the jth location. Genetic gain was estimated for all 36 combinations of selection/response locations for the two traits.

The indirect gain in each of the six locations, by selecting based on the average of the six locations, was also estimated as:

$$G_{j(M)} = H^2 SD_{j(M)}$$

where, $G_{j(M)}$ is the genetic gain in the jth location, by selection on the average of all six locations; H^2 is the broad-sense heritability based on the joint analysis of all six locations; and $SD_{j(M)}$ is the selection differential in the jth location, given by the difference in means of the clones in the jth location, whose superiority was evidenced on the average of all six locations, and the overall mean in jth location.

In all simulated selection schemes, gains were predicted considering a selection of 10% of the clones (3 clones out of 30 available). The percent gain, relative to the population mean, was estimated as:

$$pG_{j(M)} = [G_{j(M)} / M_j] \times 100$$

where, $pG_{j(M)}$ is the percent genetic gain in the jth location, by selecting on the average of the six locations and M_j is the overall mean of the trial on the jth location.

A third type of gain was the direct gain, based on selection on the mean of the six environments, given by:

$$G_M = H^2 SD_M$$

where, G_M is the direct genetic gain by selection on the mean of all six locations; H^2 is the broad-sense heritability based on the joint analysis of all six locations; and SD_M is the selection differential given by the difference in means of the clones across locations, whose superiority was evident based on the average of all six locations, and the overall mean in all six locations.

The percent gain, relative to the population mean, was estimated as:

$$pG_M = [G_M / M_M] \times 100$$

where pG_M is the percent genetic gain by selection on the average of the six locations and M_M is the overall mean of all trials.

For the estimation of the heritabilities, variance components were estimated by Restricted Maximum Likelihood (REML), using SAS. Genetic correlation between the same trait measured in different locations (type B genetic correlation) (Bourdon 1977) was estimated as:

$$r_B = \frac{\sigma^2_C}{\sigma^2_C + \sigma^2_{CxE}}$$

where, r_B is the type B genetic correlation defined as the genetic correlation between the same trait measured in two environments; $\sigma^2_{C(i)}$ is the among-clone variance component; and σ^2_{CxE} is the clone-by-location interaction. When r_B is close to zero, the clone-by-location interaction is high; while r_B is close to one the interaction is low.

Correlation between the type B correlations and the differences between pairs of locations in temperature, rain, relative humidity, latitude, longitude, soil pH and soil nutrient levels and the geographical distance of locations was estimated, aiming to identify potential causes of genotype-by-environment interaction. Also, stepwise multiple regression was undertaken.

Results and Discussion

Micro-environmental effects

The broad-sense heritabilities for the number of vegetative brooms per tree were higher than those for number of pods at the six locations (Figure 1). When 20 trees per clone were used, the heritabilities within location for the number of brooms per tree ranged from 0.91 to 0.98 (Table 2), indicating that the effect of the micro-environment on this trait is low, even considering the genetic differences among rootstock plants and the differences in soil and shade.

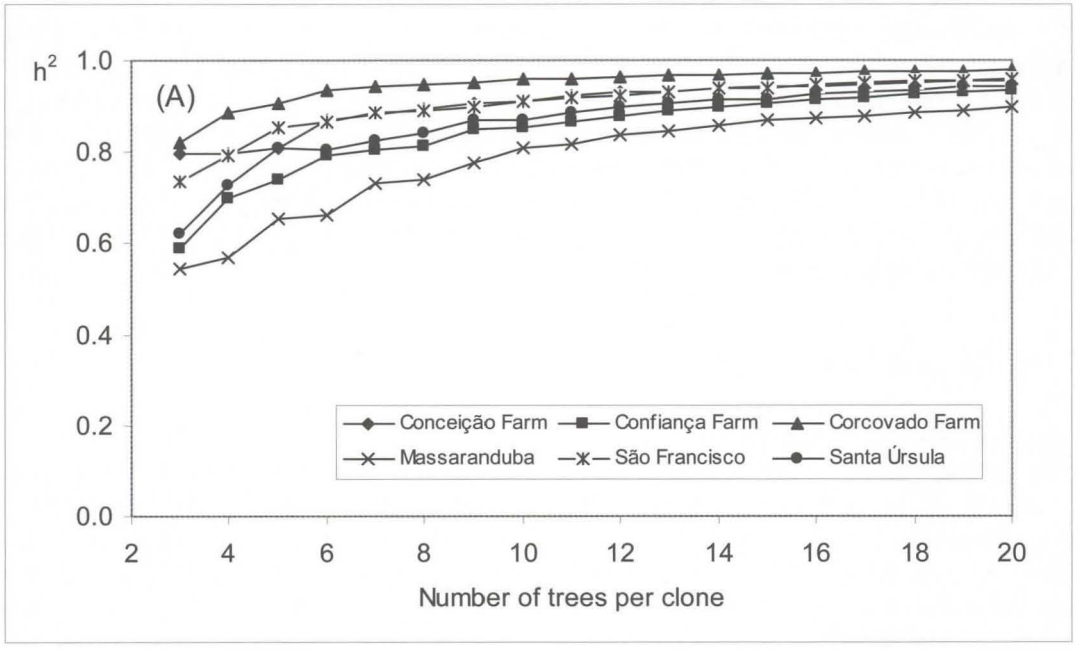
This might also suggest a low impact of the rootstock on the witches´ broom resistance of the scion clone, since some of the adult trees are likely descendants of resistant clones, such as Scavina (SCA) 6. The broad sense heritabilities for the number of pods, on the other hand, are lower (h^2 = 0.78-0.94), but still high enough to allow high genetic gains by selection on this trait (Table 2).

For both traits, there is little increase in heritability when more than 10 trees per clone are used, in most locations studied (Figure 1). For the number of brooms, no extra benefit exists in using more than 6 trees per clone to rank them (Figure 2). As few as 6 trees per clone provide the same ranking of the clones as 20 trees, for all locations considered. For the number of pods per tree, at least 10 trees are required to produce the same ranking of the clones as when using 20 trees per clone.

In summary, 10 trees per clone per location are enough for a reliable assessment of the performance of clones and in ranking them for selection based on the number of brooms and number of pods per tree. However, it should be noted that the data used for these analyses are still preliminary and caution is advised until these results can be confirmed.

Macro-environmental effects

The interaction between clones and locations was highly significant for both traits (Table 3) and the genetic correlations between pairs of locations for pod number were generally low, except for some pairs of locations (Table 2). Therefore, the best clones in a location may not be the same as those in other locations for that trait. For the number of brooms, on the other hand, the correlations were generally high ($r_B \geq$ 0.68), indicating that for this trait there is a good agreement between the ranking of the clones in different locations.



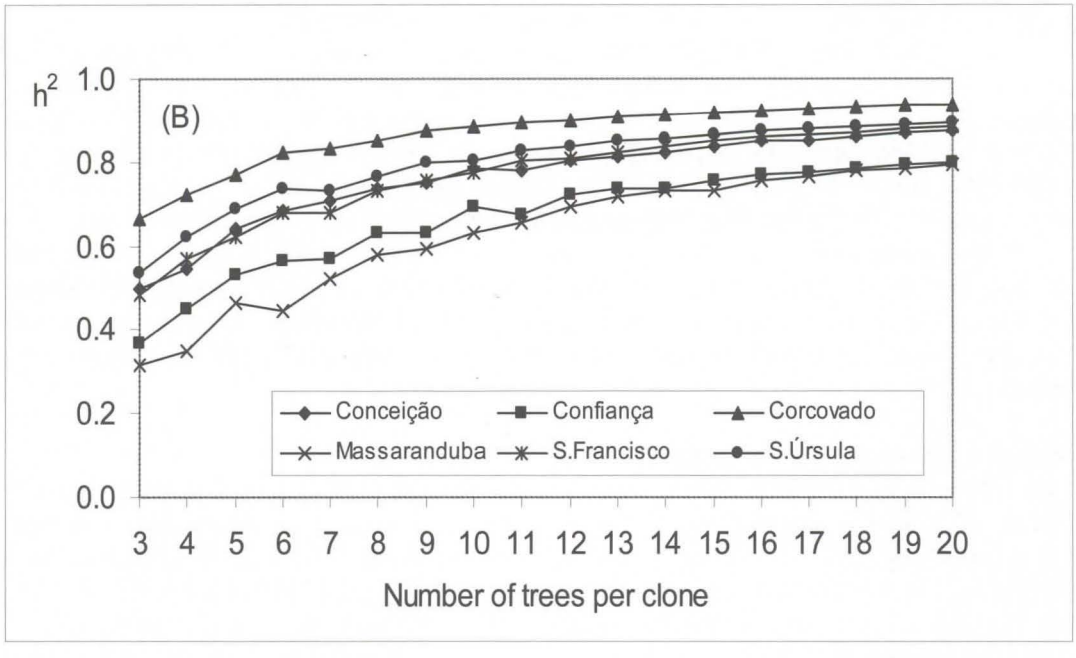


Figure 1. Broad-sense heritability for number of vegetative brooms (A) and number of pods (B), in six locations, when 3 to 20 trees are randomly picked from the 20 available

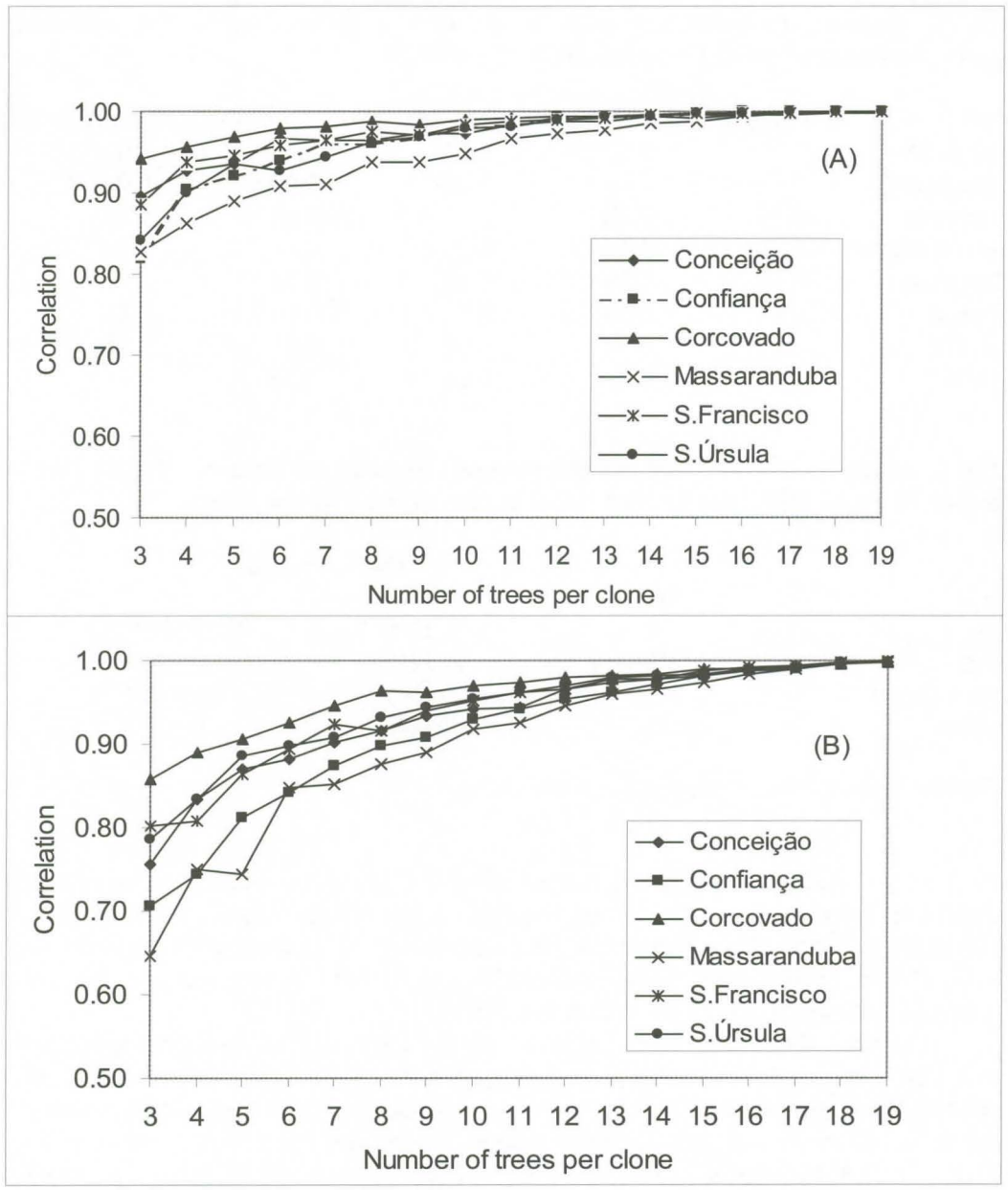


Figure 2. Pearson correlation between the number of vegetative brooms (A) and number of pods per tree (B) estimated using 3 to 19 trees per clone, with that estimated based on 20 trees per clone. The 3 to 19 clones were picked at random 30 times with replacement. All correlations were highly significant ($p < 0.0001$)

Table 2. Genetic correlation for the number of vegetative brooms (above diagonal) and number of pods (below diagonal) measured in a pair of farms (type B genetic correlation) and broad-sense heritabilities for number of vegetative brooms (NVB) and number of pods (NP)

Farm	Conceição	Confiança	Corcovado	Massaranduba	S.Francisco	S.Úrsula
Conceição	-	0.97	0.68	0.83	0.82	0.78
Confiança	0.74	-	0.85	0.98	0.95	0.96
Corcovado	0.52	0.26	-	0.93	0.95	0.99
Massaranduba	0.79	0.71	0.25	-	1.00	1.00
S.Francisco	0.45	0.29	0.84	0.26	-	1.00
S.Úrsula	0.84	0.68	0.51	0.61	0.56	-
H^2 (NVB)	0.96	0.94	0.98	0.91	0.96	0.95
H^2 (NP)	0.88	0.80	0.94	0.78	0.88	0.90

Table 3. Analysis of variance for the number vegetative brooms (NVB) and number of pods (NP), measured in 30 cacao clones in six farms

		Mean Square	
Source	**DF**	**NVB**	**NP**
Farm	5	2.36***	1361.56***
Clone	29	44.94***	192.58***
Farm x Clone	145	1.10***	33.63***
Residual	3123	0.49	6.37

*** Significant according to the F-test ($p < 0.0002$)

Aiming to understand the factors determining the clone by environment interaction, correlations were estimated between the type B genetic correlations (an indication of the interaction) and the differences between pairs of locations for several factors (Table 4). All correlations were non-significant ($p > 0.05$), except for the level of calcium at a depth of 20-40 cm within the soil.

It can be seen also from Table 4 that the distance between the test locations is not correlated with the type B genetic correlation for both traits ($r = 0.16^{ns}$ for number of brooms and 0.09^{ns} for number of pods). Therefore, when establishing recommendation zones, grouping locations closer together might not be a good strategy, since locations closer to each other can result in more genotype-by-environment interaction than locations farther apart.

Stepwise regression of all factors measured, on the type B correlations, for the number of brooms did not result in any reasonable model (not shown). All regression coefficients were significant only at the 0.15 probability level and the determination coefficient was low. On the other hand, for the number of pods, the level of calcium at a depth of 20-40 cm within the soil explained 40.4% of the variation in the interaction (Table 5) and the regression coefficient was highly significant. Since this effect was more pronounced at a depth of 20-40 cm compared to one of 0-20 cm in the soil, it is likely to be related to the fact that the 20-40 cm measurement is more representative of the basic fertility of the soil (less affected by fertilisation and/or organic matter accumulation). Therefore, based on the results found here, genotype-by-environment interaction can be minimised by a good programme of soil fertility correction, particularly at a soil depth of 20-40 cm.

Table 4. Pearson correlation between the type B genetic correlations for number of vegetative brooms ($r_{B(NVB)}$), number of pods ($r_{B(NP)}$) and several geographic, climatic, and soil factors, as well as with the average NVB and NP

Category	Variable	$r_{B(NVB)}$	$r_{B(NP)}$
Geographic	Latitude	0.16^{ns}	0.08^{ns}
	Longitude	0.17^{ns}	-0.10^{ns}
	Distance	0.16^{ns}	0.09^{ns}
Climate	Rainfall	-0.02^{ns}	0.14^{ns}
	Temperature	0.37^{ns}	-0.14^{ns}
	Relative Humidity	0.04^{ns}	0.07^{ns}
Soil	pH (0-20 cm)	0.29^{ns}	-0.20^{ns}
	pH (20-40 cm)	-0.09^{ns}	0.34^{ns}
	Al (0-20 cm)	0.14^{ns}	-0.25^{ns}
	Al (20-40 cm)	0.25^{ns}	-0.16^{ns}
	Ca (0-20 cm)	0.16^{ns}	-0.11^{ns}
	Ca (20-40 cm)	0.23^{ns}	-0.64^{**}
	Mg (0-20 cm)	0.34^{ns}	0.07^{ns}
	Mg (20-40 cm)	0.22^{ns}	-0.15^{ns}
	K (0-20 cm)	0.24^{ns}	0.13^{ns}
	K (20-40 cm)	0.12^{ns}	0.07^{ns}
	P (0-20 cm)	0.02^{ns}	0.13^{ns}
	P (20-40 cm)	0.05^{ns}	0.02^{ns}
Others	Average NVB	0.13^{ns}	0.04^{ns}
	Average NP	0.11^{ns}	0.09^{ns}

Table 5. Regression coefficients, determination coefficient and p-values associated with the effect of calcium at 20-40 cm in the soil on the type B genetic correlation for number of pods measured in pairs of locations in six cacao clonal trials

Independent variable	R^2 (%)	Regression coefficient	p-value
Intercept	-	0.77	0.0001
[Ca] 20-40 cm	40.4	-0.17	0.0109

Overall, the direct gains were higher than the indirect ones (Table 6). The highest percentage of direct gain in resistance to witches´ broom occurred in Corcovado farm and the lowest in Massaranduba farm. For this trait, selection in any farm results in high gains in any other farm; except selection in Massaranduba, which resulted in low gain in Confiança farm (Gain = 51.17%, Table 6). Also, selection based on the average witches´ broom across all locations resulted in a gain as high as the direct gain from selection in specific locations.

Considering the levels of gains observed here, in situations where resources are limited, one or a few farms could be used to evaluate resistance. In this case, Conceição and Santa Úrsula farms should be used, since they produce the largest average gains across locations.

Table 6. Direct (diagonal) and indirect (off-diagonal) genetic gains, as a percentage of the population mean, on the number vegetative brooms and number of pods, resulting from selection in one of the six locations or from selection on the average of all six locations. Three clones were selected out of 30, in all cases

Trait		Response in:						
	Selection in:	Conceição	Confiança	Corcovado	Massarandub a	S.Francisco	S.Úrsula	Average
Broom Number	Conceição	95.82	75.48	94.94	88.99	89.33	90.92	-
	Confiança	86.74	92.83	87.24	71.80	93.97	90.12	-
	Corcovado	89.73	76.80	97.74	87.50	94.09	84.03	-
	Massaranduba	84.91	51.17	76.45	90.57	36.99	71.97	-
	S.Francisco	79.79	85.55	88.97	90.57	96.35	82.72	-
	S.Úrsula	88.05	79.47	89.34	84.34	96.35	93.97	-
	Average	95.82	87.35	96.37	89.08	96.35	92.92	95.77
	Direct-Average	0.00	5.48	1.37	1.49	0.00	1.05	-
Pod Number	Conceição	110.00	76.01	88.95	139.33	103.19	100.66	-
	Confiança	64.74	92.27	78.52	65.88	117.83	80.64	-
	Corcovado	41.48	48.26	123.31	21.21	98.27	92.93	-
	Massaranduba	101.89	78.69	98.65	155.90	95.89	86.62	-
	S.Francisco	31.99	52.05	65.00	25.72	135.02	93.96	-
	S.Úrsula	67.74	38.80	108.17	51.78	104.60	140.84	-
	Average	105.29	89.55	100.89	150.36	114.06	95.31	79.08
	Direct-Average	4.71	2.72	22.42	5.54	20.96	45.53	-

For the number of pods, the largest percentage of gain over the average occurred for Massaranduba farm. In contrast to the findings for resistance, selection for the number of pods in a given location can result in poor gain in other locations. For example, selection in Corcovado or in São Francisco farm resulted in a very low gain in Massaranduba (21.21 and 25.72%, respectively), which is very low compared with the gain achieved by selecting directly on the Massaranduba farm (155.90%). Also, in contrast to selection for resistance, selection for production based on the average of locations might result in poor gain in some of those locations (*e.g.* Santa Úrsula and Corcovado). Therefore, selection based on the average of locations may not maximise the gain in individual locations.

Conclusions

- Ten trees per clone result in a genetic gain similar to that obtained using 20 trees for yield and resistance to witches´ broom, at least in these preliminary analyses.
- Grouping locations based on their geographic proximity might not be a good strategy for matching clone/recommendation zone.
- Soil factors are more important than climatic factors in causing genotype-by-environment interaction.
- Selection based on the performance at individual locations or on the average of locations results in high gains at specific locations for witches´ broom resistance, but for pod production, selection based on the average can result in sub-optimal gains in some locations.

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An Evaluation of 22 Clones at Two Locations Each with Three Planting Densities in Indonesia

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Abstract

Trials were planted at Rambong Sialang Estate in Northern Sumatra and Treblasala Estate in East Java in December 1994 to further prove some preliminary clonal selections from a range of Malaysian crosses and crosses between Bah Lias clones grown on Bah Lias Estate in North Sumatra. The Sumatra trial had 35 entries: seven control clones (four Bah Lias selections, GC29, PBC159 and SIAL93) and 28 further proof clones. The Treblasala trial had 25 entries, including the same seven controls as at Rambong Sialang, 15 of the further proof clones and three other proof clones. Twenty-two clones were common to the two trials. Both trials were planted at three planting densities: 800, 960 and 1,120 trees ha^{-1} at Rambong Sialang and 889, 1,111 and 1,333 trees ha^{-1} at Treblasala. Girth data for 1997 and 1999 were available from Rambong Sialang and annual data from Treblasala. Annual yields as weights of wet beans per plot were available for 2000 – 2002. Data were analysed for the two trials separately and then over locations, treating 800 and 1,120 trees ha^{-1} at Rambong Sialang and 889 and 1,120 trees ha^{-1} from Treblasala as lower and higher planting densities, respectively. The variates were girth, yield over the 2000 – 2002 period assuming a wet to dry weight conversion ratio of 33%, and harvest efficiency based on girth increments for the trials separately and the 1999 girths for the analysis over sites.

The Rambong Sialang trial established poorly, with 23% of the trees missing two years after planting. Coefficients of variation were high. There were significant main effects of clones on all traits evaluated (girth, yield and harvest efficiency per plot area), with increasing values at higher planting density for girth and yield. When harvest efficiency was analysed on a yield per tree basis, there was no effect of planting density. There was no evidence of clone x planting density interaction in any of the analyses.

The Treblasala trial established well with only 1.4% of the original trees lost in the first two years. There were significant effects of both clones and planting densities for all traits, including harvest efficiency based on single-tree yields which was highest at intermediate planting density. As at Rambong Sialang, there was no evidence of interaction between clones and planting densities.

In the analyses over sites, there were large effects of clones for yield, girth and harvest efficiency and smaller ones for sites, especially for girth and harvest efficiency.The interaction between sites and clones was highly significant for all traits, and often the mean square of the interaction effect was larger than that for clones. There were large effects of planting density on yield, with interactions between sites and planting densities. Planting density did not affect girth or harvest efficiency, but both showed interactions with sites. There was very little evidence of clone x density interaction. It is likely that the interactions between planting density and sites arose from the high experimental error in the Rambong Sialang trial, rather than a genetic effect. Nonetheless, a Finlay-Wilkinson analysis did show that some clones are adapted to both the environments, which is consistent with experience elsewhere.

Overall, none of the further proof clones outperformed the controls. It is suggested that firstly, better results would have been obtained had the source families been of higher performance, and secondly, the resources expended in evaluating a limited number of clones

at two locations and three planting densities would have been better used to test many more clones in observation plots at one site only.

Introduction

Growing clonal cocoa to exploit genetically superior trees was suggested by Hinchley Hart (1909) nearly 100 years ago. Despite early interest in clone selection, for example Van Hall (1930) and Pound (1936), almost all of the world's cocoa is seedling. The principal reason for this is the 1938 conclusion (cited by Posnette, 1943) that clones were inappropriate planting material for smallholder producers in West Africa. There was a resurgence of interest in clones in the seventies from W.E. Freeman (unpublished) in Trinidad and especially R. Shepherd in Malaysia (Chong and Shepherd 1986), with the recognition that clones are the best possible way of exploiting genetic variation. However, in general, cocoa breeders were slow to recognise that better clones are the basis of better seedling varieties, even if they could not be used directly as planting material. It is arguable that throughout the cocoa growing world clone selection should have received more attention (Lockwood 2003).

There is potential to treat cocoa as an orchard crop to be grown at a high planting density (Posnette 1982), say 3,000 trees ha^{-1}. Posnette thought that the development of sustainably high-yielding, high density plantings was dependant on the development of dwarfing rootstocks or the use of growth regulators. He pointed out Wood's (1964) conclusion from a survey of the literature that $2.3m^2$ was the best spacing for West African Amelonado, and $3m^2$ for more vigorous seedling types. However, little work has been done on the spacing of clonal cocoa. Mooleedhar and Lauckner (1990) demonstrated an interaction between genotype and planting density for three Trinidad Selected Hybrid clones grown at 748, 1,495 and 2,990 trees ha^{-1}. Lockwood and Pang (1996) showed that some clones are adapted to "conventional" planting density (1,096 trees ha^{-1}), others to around 3,333 trees ha^{-1} (*i.e.* Posnette's high density) and some were able to adapt to either density. Recently, Efron, Epainu, Marfu and Mombi (2003) showed differential responses of large and intermediate sized trees to planting density. Getting the planting density right is a critical component of a clone evaluation programme. Given the wide genetic variation in adaptation to planting density, are dwarfing rootstocks an imperative?

There is little information on genotype x environment interaction for clonal cocoa. Martin and Lockwood (1979) interpreted an analysis of published data from clone trials in the Caribbean as responsiveness to environment. However, field experience with the Prang Besar (PBC) clones in Malaysia was that those that performed well in Peninsular Malaysia did not necessarily do so in Sabah and vice versa. There is need for much greater understanding of genotype x environment interaction for both location and husbandry regime.

Plantation operations like PT PP London Sumatra Indonesia are well able to adopt clonal technology in cocoa, provided that they have access to proven high performance clones. A clone selection programme based on two-stage evaluation was started in 1987 (Redshaw and Zulnernin 1994). High yielding trees were identified in progeny trials at Bah Lias, with family performance (Simmonds 1996) taken into account: 10% of seedlings were selected from the first selected progenies and 5% from second selected ones. Some ortets resulted from mass selection in commercial fields planted with commercial seed from Harrisons Malaysian Plantations Bhd's Bagon Datoh Estate. Altogether 524 ortets were evaluated in preliminary trials 332 and 342 at planting densities of 750 and 842 trees ha^{-1}, respectively. These trials were planted in 1988 and 1989, using small plots and two or three replications. Thirty-one clones that yielded 150 – 200% of PA 121 were further evaluated in trials at two widely separated sites, both at three planting

densities. This paper discusses the results of this second stage evaluation and the implications for clone selection procedures in cocoa.

Materials and methods

The two trials (Table 1) were planted in December 1994. One of them was planted on Rambong Sialang Estate in North Sumatra on a yellow-brown podsolic soil following oil palm. Shade was provided by coconuts (37/ha) with *Gliricidia sepium* and *Crotolaria anagyroides* as temporary shade. The other was planted on Treblasala Estate in East Java on a latosol that had previously carried rubber. Permanent shade was provided by coconuts (37/ha), and temporary shade by *G. sepium* and *Flemingia macrophylla*. Replacement of missing points (plants) continued for two years with planting of seedlings for field budding. Husbandry practices corresponded to those on the estates with a full programme of weeding and pest management, mostly to control *Helopeltis theivora* at Treblasala in 1997–98 and sporadically at Rambong Sialang, and cocoa pod borer (*Conopomorpha cramerella*) at Rambong Sialang from October 1995 and Treblasala from May 2002. VSD was more prevalent in North Sumatra than in East Java.

Table 1. Experimental details of trials at Rambong Sialang and Treblasala

	Rambong Sialang	Treblasala
Clones	35	25
Spacing between rows (m)	3.6	3.0
Spacing within rows (m)	3.47 (800/ha) 2.89 (960/ha) 2.48 (1,120/ha)	3.75 (889/ha) 3.0 (1,111/ha) 2.5 (1,333/ha)
Trees/plot	20	25
Replications	4	3

Thirty-five clones including controls were planted in the Rambong Sialang trial and 25 in the Treblasala one. Twenty-two clones were common to the two trials (Table 2). The controls were the introduced clones GC 29 and SIAL 93, PBC 159 from Shepherd and Chong's Prang Besar selection programme in Peninsular Malaysia, and Bah Lias selections 9, 649, 679 and 2936 that were high yielding in earlier trials. The experience in East Malaysia was that when planted at 1,096 trees ha^{-1}, PBC 159 and "SIAL" 93 (probably the same clone as at Bah Lias) yielded about 60% of BR 25, the highest yielding clone in East Malaysia. SIAL 93 yielded more than 200% of PA 121 (Pang Thau Yin, unpublished observations).

Girths were measured as circumferences, in cm, 15cm above ground level in January 1997 and October 1999 in the Rambong Sialang Trial and at approximately twelve-month intervals between September 1998 and June 2002 in the Treblasala trial.

Yields were recorded as weights of wet cocoa per plot from the commencement of bearing until the end of December 2002. Yields of wet beans per plot for 2000, 2001 and 2002 were converted to dry weight equivalents using a factor of 0.33 and analysed separately and over the three years pooled.

Harvest efficiency (Daymond *et al.* 2002), the ratio of crop to (increasein) trunk cross-sectional area, was estimated in both trials. Five sets of girth data were available from the Treblasala trial and two sets from the Rambong Sialang trial.

Harvest efficiency was estimated from the increase in trunk cross-sectional area for both trials, 1997 to 1999 for Treblasala and 2000 – 2002 for Rambong Sialang. However, only 1999 girth data were available from both trials, so these were used to estimate harvest efficiency for an analysis across environments. Yields were recorded over the 2000 – 2002 period. Harvest efficiency was calculated first from the plot means (area basis *i.e.* total yield per plot divided by trunk section increment per plot) and then from yield per surviving tree (tree basis, average yield per tree within plots). The second analysis was needed because of the large number of missing trees at Rambong Sialang and in a few plots at Treblasala.

Two series of analyses were undertaken. In the first, the trials were analysed separately with all their entries and planting densities. These analyses will be summarised in this paper but detailed results are not presented. In the second set of analyses, the 22 clones common to the two trials were analysed at 800 and 1,120 trees ha^{-1} (Rambong Sialang) and 889 and 1,111 trees ha^{-1} (Treblasala), so that effects of trials, clones and planting densities could be separated. The clone x environment interaction was investigated further using Finlay and Wilkinson's (1963) joint regression method.

No attempt was made to correct for missing trees in any of the analyses. All analyses were done using GENSTAT 5 version 3.2 except that SPSS version 10.1 was used for the Finlay-Wilkinson analysis.

Results

Rambong Sialang

This trial was difficult to establish with substantial mortality of planted trees. By 1997, an average of 4.56 trees per plot had been lost (22.8%), with a maximum of 16 (Table 2). However, statistical analysis showed no effect ($p>0.05$) of clones, of planting density or of the interaction between the two on mortality rates. As expected, the high mortality was associated with inflated coefficients of variation, which were 51.0% for yield in 2000 - 2002 and 41.7% for harvest efficiency calculated on a yield per tree basis.

In the analysis of the 1999 girths, there were significant effects of clones ($p<0.001$) and of planting density ($p<0.05$), but not of interaction between the two ($p>0.10$). BL 342-242 was the most vigorous clone, SIAL 93 was the third most vigorous and PBC 159 was the second weakest (Table 2). GC 29 was slightly more vigorous than PBC 159. Somewhat unexpectedly, the clones were a little more vigorous at higher planting density, as shown in Table 2.

The average yield over the three-year period 2000 – 2002 was 2.43t ha^{-1} (Table 3). In the statistical analysis, the main effects of both clones and planting densities were significant at $p=0.001$. There was no evidence of interaction between clone and planting density ($p>0.10$). BL 342-269 was the highest yielding clone at 5.39t ha^{-1}, followed by PBC159 at 4.08t ha^{-1} and SIAL93 at 3.86t ha^{-1}. The lowest yielding clone gave only 0.26t ha^{-1} over the three years. Yield per plot unit area increased with planting density:

• Planting density/ha:	800	960	1,120
• Mean yield (t/ha):	2.13	2.35	2.82 (SED 0.148)

Table 2. Tree survival and girth at Rambong Sialang and Treblasala

Clone	Original Trees per Plot 1997		Girth cm June 1999	
	Rambong Sialang (20 planted)	Treblasala (25 planted)	Rambong Sialang	Treblasala
BL2936	14.7	24.7	28.1	30.5
BL332-15	14.9	23.0	26.2	29.8
BL342-109	14.4	24.5	27.1	30.6
BL342-147	17.1	23.5	29.8	30.1
BL342-161	13.5	24.0	26.5	30.3
BL342-242	16.7	23.8	31.1	30.9
BL342-248	15.5	24.0	25.0	30.0
BL342-263	16.9	22.2	27.7	29.8
BL342-269	17.4	24.0	29.1	30.9
BL342-285	15.2	24.0	23.7	30.4
BL342-396	16.4	23.3	28.5	29.9
BL342-408	15.4	24.3	23.5	29.9
BL342-425	13.9	24.3	26.8	31.3
BL342-5	16.0	23.7	27.2	30.3
BL342-68	14.9	23.5	27.5	31.5
BL342-70	16.1	23.8	27.4	30.4
BL649	17.2	23.0	27.8	30.8
BL679	16.0	23.8	28.7	29.9
BL9	15.5	23.2	25.6	30.4
GC29	17.2	23.8	26.6	30.8
PBC159	14.5	24.0	23.5	30.4
SIAL93	17.1	23.0	29.4	30.7
Mean	15.8	23.7	27.1	30.4
SED	1.17	1.36	0.91	1.06

- Planting density/ha: 800 960 1,120
- Mean girth (cm): 26.48 26.73 27.33 (SED 0.338)

Harvest efficiency was calculated from the increment in trunk cross-sectional area from 1997 to 1999. It was estimated firstly on the basis of yield per plot unit area and then per tree. In the first analysis, the effects of both clones and planting densities were significant at p=0.001. Harvest efficiency increased with planting density. In the second analysis, only the effect of clones was significant, and harvest efficiency was highest at the lowest planting density. There was no evidence of interaction in either analysis (p<0.10). On a per tree basis, PBC 159 had the highest harvest efficiency followed by BL 342-269, with SIAL 93 third and GC 29 fourth (Table 3).

Treblasala

This trial established much better than the one at Rambong Sialang with only 1.36% of the trees missing or replaced in 1999 (Table 2). A maximum of 8 trees was lost from one plot. In a statistical analysis of mortality, there was no evidence of an effect of clones, planting density or their interaction ($p>0.10$)

Analysis of the 2002 girths showed significant effects ($p=0.001$) of clones and planting density, but not of interaction. SIAL 93 was the most vigorous clone, followed by BL 9 and BL 679 (Table 2). Only three clones were weaker than PBC 159, and as in the other trial, GC 29 was only slightly more vigorous than PBC 159. Girths were greater at lower planting density:

- Planting density/ha: 889 1,111 1,333
- Mean girth (cm): 41.7 40.7 39.5 (SED 0.221)

The average yield over the last three years was 4.38t/ha (Table 3), 1.80 times that in the other trial. SIAL 93 was the highest yielding clone with 5.68t ha^{-1}, with BL 342-408, BL 2936, BL 649 and BL 342-5 all yielding over 5t ha^{-1} over the three years. PBC 159 and GC 29 were just above the average for the trial. BL 342-269, which was the highest yielding clone in the Rambong Sialang trial, was below average at Treblasala. Yields increased sharply with planting density:

- Planting density/ha 889 1,111 1,333
- Mean yield t/ha 3.37 4.71 5.04 (SED 0.060)

with no evidence of interaction between clone and planting density ($p>0.2$).
Harvest efficiency was calculated from the growth increment over the 2000 – 2002 period, firstly using yield per unit area and then yield per tree. The main effects of clones and of densities were significant ($p=0.001$) in both analyses, with no evidence of an interaction. In the analysis based on yield per unit area, harvest efficiency increased progressively with planting density, but in the analysis based on yield per tree, the intermediate planting density had the highest harvest efficiency. BL 342-408 had the highest harvest efficiency, followed by BL 649 and BL 2936. GC 29, PBC 159 and SIAL 93 were all just above average for harvest efficiency.

Analysis over sites

Twenty-two clones were common to the two sites (Table 2). However, the planting densities differed, and the data were restricted to 800 and 1,120 trees ha^{-1} at Rambong Sialang and 889 and 1,111 trees ha^{-1} at Treblasala, which were treated as lower and higher density, respectively. The trees grew faster and were higher yielding at Treblasala and showed a slightly higher harvest efficiency (Tables 2 and 3).

In the analysis of mortality data, significant effects were detected of sites ($p<0.001$) and of planting density ($p<0.05$), but the effect of clones was not significant ($p>0.2$). This suggests that the clones did not differ in establishment ability under the conditions of the trials.

The analysis of the 1999 girth data showed a significant effect of sites ($p<0.01$) and of clones ($p<0.001$). There were significant interactions between sites and clones ($p<0.001$) and between sites and planting densities ($p<0.01$). When the effect of clones was tested against the interaction with sites, the variance ratio was 1.56 with 21/21 degrees of freedom, which is not significant at $p=0.10$, suggesting that nothing can be said about overall differences between clones.

The yield analysis gave similar results to the girth analysis. The effects of sites and clones as well as planting density and interaction between sites and clones were significant at p=0.001. The interaction between sites and planting densities was significant at p=0.05 (Figure 1). The mean square for the sites x clone interaction was

Table 3. Yield and harvest efficiency at Rambong Sialang and Treblasala

Clone	Yield t/ha 2000 – 2002		Harvest efficiency (kg dry beans /tree 2000-2002 per cm^2 of trunk cross-sectional area	
	Rambong Sialang	Treblasala	Rambong Sialang	Treblasala
BL2936	3.04	5.04	0.0530	0.0679
BL332-15	1.28	4.02	0.0242	0.0568
BL342-109	2.89	3.48	0.0500	0.0467
BL342-147	3.38	2.84	0.0510	0.0392
BL342-161	1.82	3.37	0.0338	0.0461
BL342-242	3.63	3.77	0.0492	0.0493
BL342-248	3.73	3.82	0.0563	0.0537
BL342-263	3.01	3.65	0.0507	0.0516
BL342-269	5.27	3.68	0.0794	0.0483
BL342-285	2.12	4.43	0.0492	0.0602
BL342-396	4.00	4.64	0.0650	0.0646
BL342-408	0.87	5.43	0.0197	0.0768
BL342-425	1.47	3.37	0.0289	0.0432
BL342-5	2.86	4.65	0.0516	0.0635
BL342-68	3.89	3.36	0.0637	0.0428
BL342-70	3.76	3.46	0.0668	0.0471
BL649	0.21	5.06	0.0036	0.0673
BL679	1.57	4.27	0.0229	0.0593
BL9	2.81	3.14	0.0507	0.0427
GC29	3.23	4.21	0.0598	0.0558
PBC159	3.55	4.13	0.0870	0.0565
SIAL93	3.86	5.40	0.0550	0.0715
Mean	2.78	4.06	0.0487	0.0550
SED within site	0.475	0.549	0.00667	0.00770

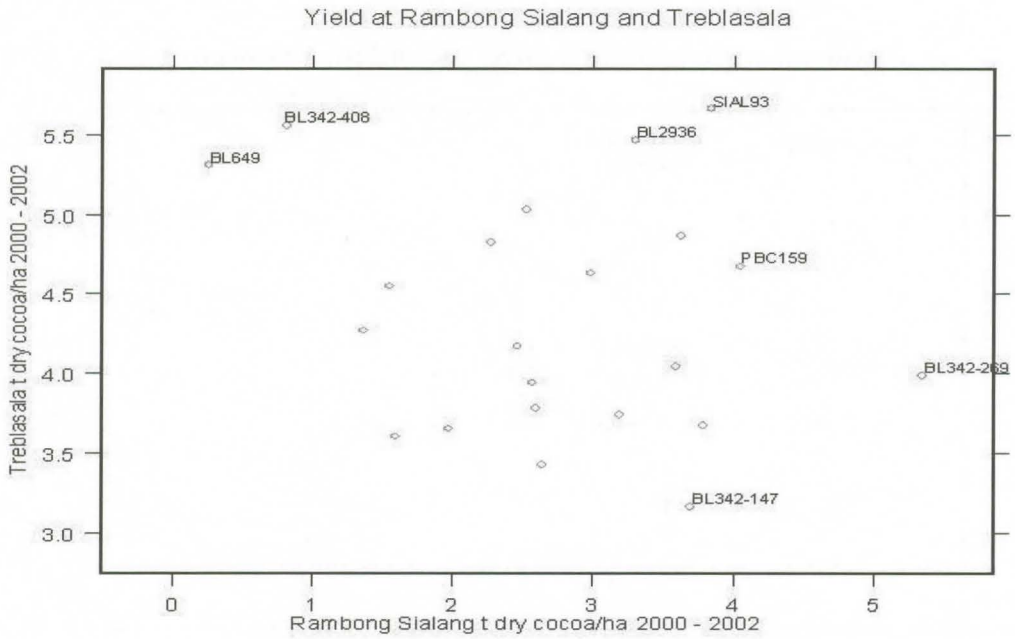

Figure 1. Yield at Rambong Sialang and Treblasala

larger than the mean square for clones, so nothing can be said about the overall differences between the clones.

Harvest efficiency was calculated using the trunk cross sectional areas in 1999, as these were the only measurements common to the two trials. In the analysis based on single trees, the effect of sites was significant at p=0.05, and that of clones at p = 0.001. However, there were highly significant interactions between sites and clones and between sites and planting densities, with the mean squares both larger than the mean square for clones. As with the girth and yield analyses, nothing can be said about differences among the clones over the two sites.

Harvest efficiency was calculated for the trials separately using the 1999 trunk cross-sectional areas. In the Rambong Sialang trial, the clone means were closely correlated with those obtained from the analysis based on growth increments, r=0.997. The correlation for the Treblasala trial was r=0.917. These correlations suggest that basing the harvest efficiency analysis on the 1999 girths did not introduce a large bias.

Genotype x environment interaction for yield

The plot of the regression coefficients from genotype x environment analysis against average genotypic values is represented in Figure 2. There is variation among the clones in their adaptation to environment. For example, clones BL 649 and BL 342-408 have high regression coefficients as a result of their high yields at Treblasala and poor yields at Rambong Sialang (Table 3). They are susceptible to vascular streak dieback disease and *Helopeltis*, respectively. BL 342-147 has a negative regression coefficient. BL 342-269 performed well in Rambong Sialang, but less so at Treblasala where it was affected by *Phytophthora* pod rot and rats. The other four high yielding clones, SIAL 93, BL 2936, BL 342-269 and PBC 159 are well adapted to the two environments.

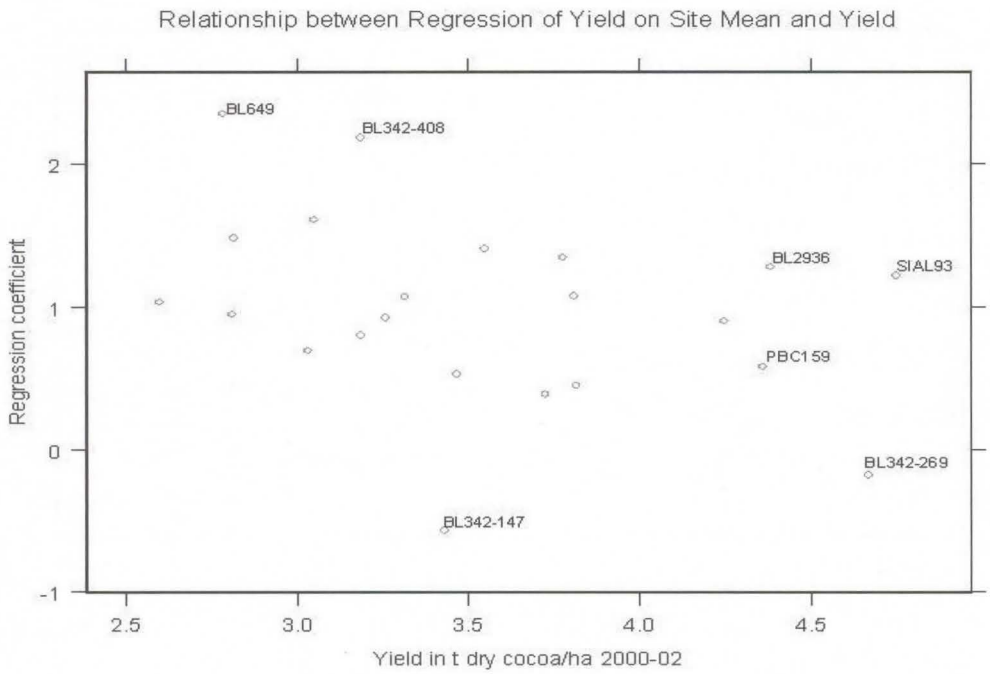

Figure 2. Relationship between regression of yield on site mean and yield

Discussion

The Treblasala trial was an excellent one with good establishment, acceptable yields and reasonably low coefficients of variation. In contrast to the trials in Trinidad and Malaysia, there was little evidence of an interaction between clone and planting density for vegetative vigour or yield: the variance ratio was significant at p=0.05 only in the analyses of the 1999-2000 yields and the growth increment from 2000 to 2002. The clones had been pre-selected for yield at relatively low planting density, and the range of planting densities in the trial differed by a factor of only 1.5, compared to 3.0 for Lockwood and Pang (1996) and 4.0 for Mooleedhar and Lauckner (1990). The clones used by the latter authors had been pre-selected for adaptation to the relatively low planting densities that were used, but Lockwood and Pang's had not. However, the possibility remains that interactions with planting density would emerge over a longer period of recording.

The results of the trials at Rambong Sialang are consistent with those from Treblasala, in that there is no evidence of interaction between clone and planting density over the narrow range of planting densities tested.

In the analyses of clone x site interactions there was a significant effect (p=0.001) of clones in all the analyses of girths, yields and harvest efficiency. The effect of sites was significant in most analyses whereas planting density was significant (at p=0.05) in only one. The interaction between sites and clones was significant at p=0.001 in all the analyses, and there were significant interactions between sites and planting densities. Given that there was little evidence of a main effect of planting densities, the latter interactions are hard to interpret and taking into account the high experimental error of the Rambong Sialang trial, few firm conclusions can be reached from the analysis over sites.

The Finlay-Wilkinson analysis showed that some clones are adapted to both environments, which is consistent with Martin and Lockwood's (1979) conclusion and with the widespread adoption of some of W.E. Freeman's clones in Brazil. If cocoa clones with reasonably broad adaptation are readily available, clone selection programmes can be based on initial selection at a single site, with judicious choice of planting density. As promising clones emerge, they will be evaluated at a wider and wider range of sites on an increasing scale as confidence in them grows, always at appropriate planting density. It would be unacceptably risky to grow clones on a large scale in new environments or from new breeding populations in known environments without proving them first. The proving process also provides a multiplication phase and will minimise the risk to farmers. In addition, it will allow the potential for genotype x environment interactions to be exploited by selecting clones for specific environments.

The two trials, totaling 15ha to evaluate 31 clones, represented a considerable investment. None of the further proof clones consistently outperformed the controls, suggesting that little if any genetic advance was secured. If Simmonds' (1996) theory holds, lack of advance at the family level was a major contributor to this disappointing outcome.

It is further arguable that little was gained from the use of a narrow range of three planting densities: the lack of interactions suggests that the preliminary selection for adaptation to "normal" planting density had been broadly successful. The important question of interaction between clone and site remains largely unanswered.

Acknowledgements

We thank the Bah Lias Research Station breeding staff in North Sumatra and East Java who carried out the field trials. This paper is published with the permission of the Board of PT PP London Sumatra Indonesia Tbk.

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High Density Planting of Cacao: the Trinidad and Tobago Experience

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Abstract

Cocoa is an important agricultural commodity in Trinidad and Tobago. However annual production of fine or flavour cocoa has declined to 1.5m tonnes in the last five years. High density planting (HDP) was introduced in the 1980's as an alternative technology to traditional systems that are based on low density planting (LDP, normally at 748 plants ha^{-1}). Trials conducted in Trinidad showed higher yields being obtained from three commercial Trinidad Selected Hybrid (TSH) clones, at 6-8 years after planting, at densities of 2990 trees ha^{-1} than at 748 and 1495 trees ha^{-1}. A significant variety x spacing interaction pointed to differences in clone behaviour, with the less vigorous TSH 919 clones performing best at the highest density. Data from other field trials also showed better performance of TSH clones and TSH-derived open-pollinated seedling progenies at HDP in relation to LDP.
A successful commercial HDP farm in east Trinidad has achieved yields of 1500 kg ha^{-1} in its 11^{th} year of production. The variable cost of production per ha for improved LDP and HDP is US$ 806 and US$1699, respectively, which is indicative of the higher management levels required for HDP. Agronomic factors contributing to productivity on this farm are also examined. Adoption of the HDP system is recommendable based on a higher benefit: cost ratio than for LDP. The performance of Trinidad's cocoa industry can therefore be expected to improve based on the further development of HDP. The reasons that the adoption rate of HDP technology in Trinidad is still very low among small farmers are discussed.

Introduction

Cocoa is one of the commodities that contribute to export earnings in the agricultural sector of Trinidad and Tobago. Trinidad was the fifth largest producer in the world during the 1920's. Yields peaked at 34,000 tons in 1921, and have declined steadily to approximately 1.5 metric tonnes in 2000.

The local industry is comprised of about 3000 small farmers, each operating on less than 10 ha, and 20 to 30 large farmers who produce on 20 to 100 ha of land each. All farmers commonly use low density planting (LDP) of cocoa at 3.0 x 3.0 to 3.8 x 3.8m with shade trees (*Erythrina* spp) at 10 to 15 plants ha^{-1}. Smallholders often favour companion cropping with bananas and other fruit species and use little or no fertilisers and fungicides. Black pod disease losses can be as high as 40% under these conditions (Shripat, 2001), and productivity ranges from 150 to 400 kg ha^{-1}. On the larger farms, higher inputs are used and yields range from 400 to 700 kg ha^{-1}.

In the1980's and 90's, high density planting (HDP) technology was researched and developed by the Ministry of Agriculture, Land and Marine Resources (MALMR), as an alternative to the traditional LDP systems. HDP seeks to optimise yield per unit area of land (Cortes and Perez 1986). In HDP, the yield per plant is lower but the cumulative yield for a much larger plant population is significantly higher. In cocoa, the optimum spacing between trees is the planting density which will give the greatest economic return per unit area of land (Armstrong

1976). The last decade has seen the emergence of five large farms, which have adopted HDP on over 200 ha in Trinidad.

This paper reviews the formal research on HDP, examines its application in a commercial cocoa enterprise setting in Trinidad, explains its low rate of adoption among small farmers and develops an economic understanding of both HDP and LDP systems within the Trinidad and Tobago context.

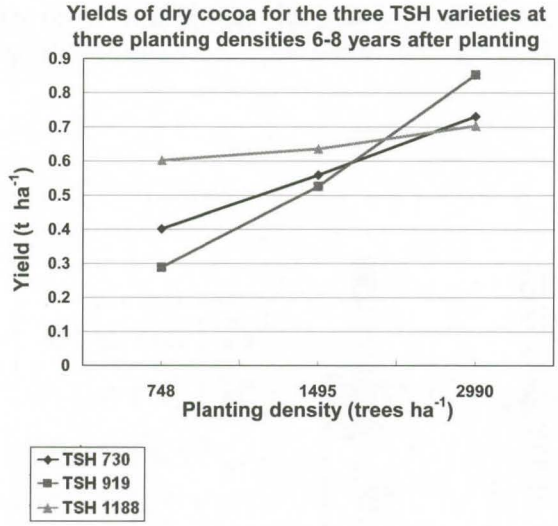
HDP technology development

The low productivity and profitability of cocoa farms in the 1970's prompted a closer look at various aspects of cocoa growing. This resulted in the proposal by the late W.E. Freeman for a new approach to cocoa growing (Freeman 1975). This new approach focused on the reduction of spacing from 3.6 x 3.6m to 1.8 x 1.8m, minimum permanent overhead shade, reduced levels of pruning and the use of fertilisers. This HDP system was found to be superior to the traditional system (LDP) from trials conducted at the Research Section of MALMR.

Mooleedhar and Lauckner (1990) investigated the effect of three spacing treatments: (1) traditional planting distance of 3.6 x 3.6m (748 plants ha^{-1}), (2) an intermediate spacing of 3.6 x 1.8 m (1495 plants ha^{-1}) and (3) a close spacing of 1.8 x 1.8m (2990 plants ha^{-1}), on the yield of three commercial Trinidad Selected Hybrid (TSH) clones (TSH 730, 919 and 1188). The authors analysed yields for the initial production years, between 1985 and 1987. The yields obtained from the close spacing were significantly better than those from the intermediate and traditional spacings. Differences in clones were only observed in 1987, with TSH 1188 yielding best (Mooleedhar and Lauckner 1990). This was attributed to the high rainfall in 1987 (TSH 1188 being more resistant to black pod). A significant ($P<0.01$) variety by spacing interaction was observed for average annual yields over the period. TSH 1188 performed best at the traditional spacing while TSH 919 appeared best suited for closer spacing (Figure 1a). The greater adaptability to wider spacing of TSH 1188 is due to its characteristic high early vigour for which it was selected.

Shripat and Bekele (1996) continued to study the trial described above and analysed yields for a further three years, between 1988 and 1991. Average annual yields were found to increase linearly as spacing between plants decreased, with again a significant effect of planting density for each of the recorded years. The main effect of clones was significant only in 1990, together with a significant clone by spacing interaction also only in this year (Shripat and Bekele 1996). Similar yields were obtained for all clones at the widest spacing, while TSH 919 performed best at the close and intermediate spacings (Figure 1b). The relatively lower yields of TSH1188 over the second period was attributed to its relative susceptibility to the severe drought that occurred in 1989, while TSH 919 showed good drought tolerance. At the end of the six-year observation period, annual yields had reached 1.5 tonnes ha^{-1} and average yields were still increasing.

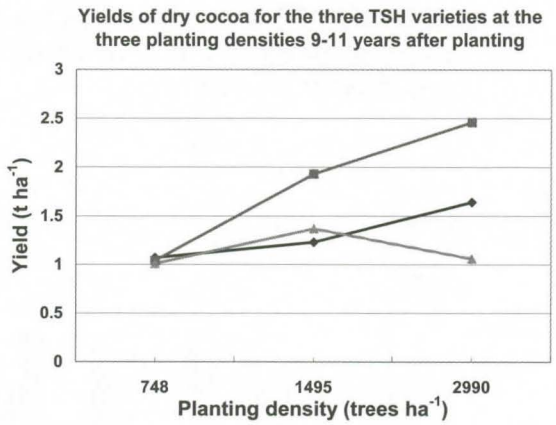
Several other trials were conducted in Trinidad and have demonstrated the superiority of close spacing over traditional spacing. The results of some of these trials were reported by Mooleedhar (1986). In Table 1, the results of one trial on the performance of clones and seedlings under traditional and close spacing is reported. This trial was carried out because farmers in Trinidad usually have particular preferences for either clonal or seedling planting material. The results show much better performance of clones as well as of seedling progenies at HDP (Table 1).



SEM = ± 0.035 (104 d.f).

Figure 1a. Yields of dry cocoa for the three TSH varieties at three planting densities 6-8 years after planting

Source: Mooleedhar and Lauckner (1990)



SED of any two cultivar means = 0.13
SED of any two cultivar x spacing means 0.22

Figure 1b.Yields of dry cocoa for the three TSH varieties at three planting densities 9-11 years after planting

Source: Shripat and Bekele (1996)

Table 1. Mean yields of dry cocoa (kg ha^{-1}) for clones and seedlings at two planting densities

Year	Spacing			
	1.83m x 1.83m		3.66m x 3.66m	
	Clones	Seedlings	Clones	Seedlings
1982	1016	658	210	195
1983	1046	1494	457	522
1984	1285	1225	410	343
1985	1315	1016	388	246
1986	2164	1734	473	470
Means	1365	1225	388	355

Source: Mooleedhar (1986)

In another trial, the yields of four TSH clones, planted at a density of 2990 plants ha^{-1}, were assessed over an eight-year period. The results of this trial show that maximum yields of 2000 to 2500 kg of dry cocoa can be obtained with the best clones at 8 – 11 years after planting (Figure 2).

The major conclusions drawn from the aforementioned trials are that:

- HDP with a population of 3000 plants ha^{-1} consistently out yielded LDP. Yields can approach 2500 kg ha^{-1} for HDP with 9 to 11 year-old trees. The high yields due to HDP can therefore be sustained up to the eighth year of production.
- Both seedlings and clones performed significantly better at HDP. The improved clonal material represented by the TSH varieties performed significantly

better at HDP than at LDP. Differences exist however in the performance of clones at different planting densities. This can be attributable to environmental conditions or inherent vigour.

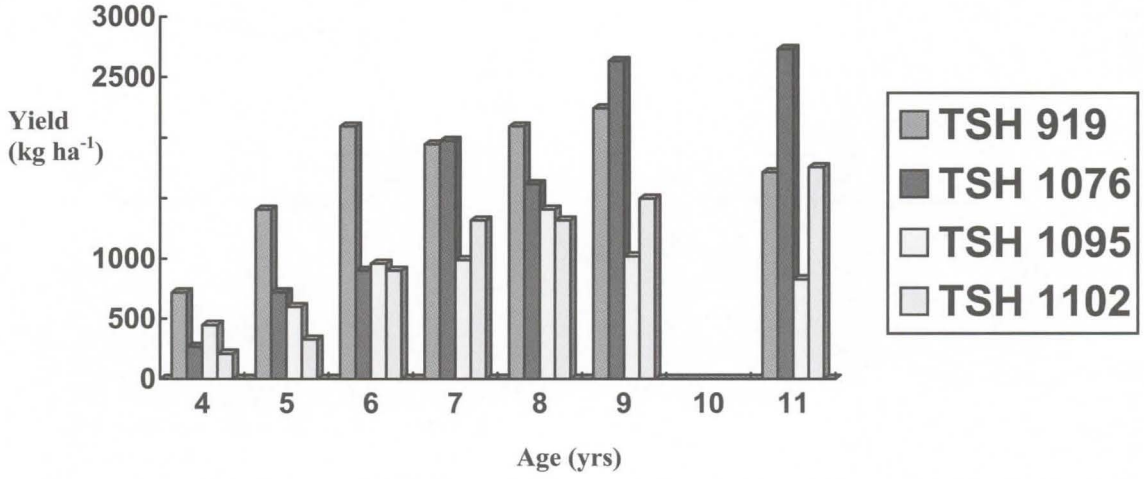


Figure 2. The long-term effect of close spacing on the yields of four TSH clones

Source: Mooleedhar (1986)

Reasons for superiority of HDP

Mooleedhar (1986) cited two major reasons for the superior performance of HDP over LDP. These relate to the plant population and to the plant environment. Firstly, HDP has four times as many plants per unit area of land than LDP. The relative number of productive units (trees) surviving over a period of time is much greater for HDP than LDP. The sustained high level of production from a cocoa field is directly related to the number of productive trees. Assuming that the rate of decline is the same for both HDP and LDP, then the number of productive units after several years is considerably less for LDP than for HDP. Table 2 demonstrates the effect of this principle from a random sample of three trials at three different locations in Trinidad.

Secondly, under HDP, the completely closed canopy formed by the overlapping cocoa trees provides the essential features of a productive field; uniform microclimate, conditions suitable for the development of pollinating insects, and optimal water relations.

Additionally, Freeman (1975) showed that in a typical LDP farm in Trinidad the best 25% of trees produced 53% of the cocoa yield, while the worst 25% produced only 2% of the yield. Poor and missing trees are frequently observed which reduce the average yields to non-economic levels. In HDP, vigorous adjacent trees will easily fill open spaces caused by poor or missing trees. Also, serious breaks in the canopy allow the penetration of too much light, leading to weed growth and loss of soil moisture.

Table 2. A Comparison of the reduction in plant population of clones and seedlings at three different locations in Trinidad after ten years

Location	Clones					
	LDP			HDP		
	No. of pickets	% Deaths	No. of surviving plants	No. of pickets	% Deaths	No. of surviving plants
North Trinidad	302	0	302	1210	15	1029
East Trinidad	302	22	236	1210	50	605
Central Trinidad	302	12	266	1210	15	1029
	Seedlings					
	LDP			HDP		
	No. of pickets	% Deaths	No. of surviving plants	No. of pickets	% Deaths	No. of surviving plants
North Trinidad	302	0	302	1210	18	992
East Trinidad	302	20	242	1210	40	726
Central Trinidad	302	12	266	1210	21	956

Commercial application of HDP in Trinidad- A Case Study

The experience of a successful commercial farm in East Trinidad using HDP technology is described hereunder.

Background

The farm is located in East Trinidad, with mean annual rainfall of 2000 mm falling mainly between July and December (wet season) and a marked dry season from January to June, with less than 400 mm of rain. Temperatures approximate 26.7°C and the average monthly relative humidity is 81%. Average total monthly sunshine hours are 230. The soils are fine, mixed, acid Aeric Tropaquepts and the topology is flat. The acreage of the farm is 15 ha, and it was established over the period of 1991 to 1993. The planting material used was seedlings, obtained by open-pollination of commercial TSH clones (TSH 919, 1076,1095,1104 1188 and 1220), which are cross- compatible. Plants were established in a clear- felled system, with a spacing of 1.4 x 1.4m. This gives a density of 5000 plants ha-^{1} and an overall population of 75,000 plants. Shade establishment at six trees ha-^{1} of *Erythrina* spp. function mainly as a windbreak. During the early years of establishment, approximately 10% of the trees were replaced due to deaths.

Productivity

In its ninth year of production, an average yield of 1680 kg ha-^{1} was achieved. Figure 3 shows that yields may still be climbing. The farmer predicts his yields will peak around 2500 kg ha-^{1} in 2006, and will be sustained for over a ten-year period thereafter. This he feels can be achieved from fertilising and optimum field sanitation. Yields will be comprised of the following components:

- Plant population 4500-4800 trees ha-^{1}
- Number of pods per tree 10-15
- Number of beans per pod 40-45
- Average bean weight 1.2-1.4g

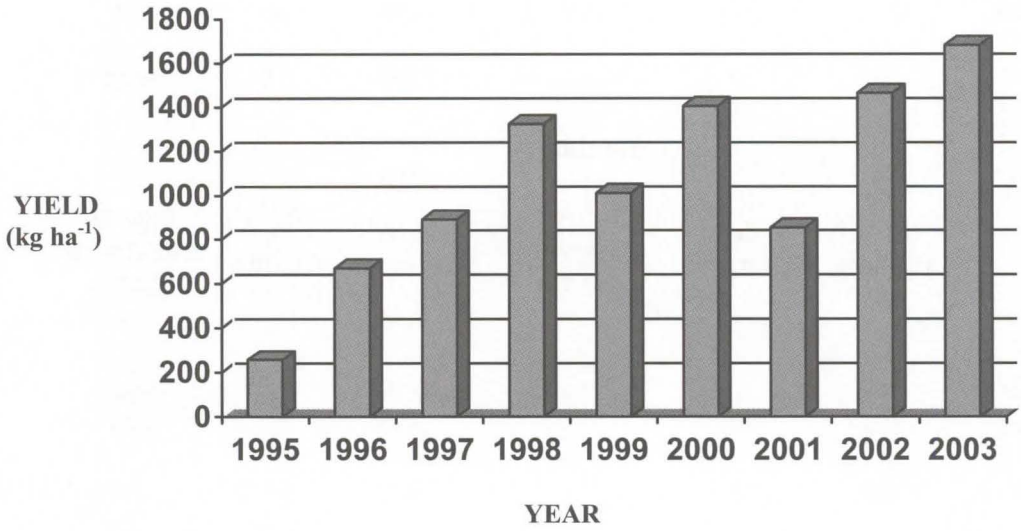


Figure 3. Annual cocoa yields (kg ha^{-1}) for a HDP farm in East Trinidad

Rainfall levels less than 400 mm during the January to June dry season could be a limiting factor on yields since no irrigation is applied. This is reflected on the yields in 2001. The root system of seedlings will cope better with this limitation than clonal material.

Agronomic practices contributing to the performance of this HDP system

Tree Management

The trees were established using a square planting distribution and rows were oriented in a North-South direction. This arrangement will give the least mutual shading and root competition (Jackson 1975; 1980). The trees are generally very uniform in size and conformation. Tree girth is now averaging 32 cm, and canopy height and width is 4.6 and 3.5m, respectively. HDP has considerably reduced the tree girth, which under LDP can be over 40 cm for TSH varieties of a similar age.

The seedling growth habit has been manipulated to give a single vertical trunk with four to five plagiotropic fan branches. This facilitated shaping and formation of the trees in the early years, while the upright growth habit allows for easy access into the fields for maintenance and harvesting operations (Glincestein *et al.* 1990). Temporary banana shade was removed in the third year after planting when the cocoa canopy closed. Pruning is done once per year to remove undesirable chupons and diseased branches. Operations are done with secauteurs and saws to minimise the destruction of flower cushions. Canopies are managed to prevent overgrowth and

self-shaded limbs. However, vegetative growth removal is kept to a minimum to reduce carbohydrate metabolism disturbance within the trees. Correct pruning practices have resulted in a favourable balance between bearing wood and canopy volumes to ensure adequate pod set and development. Heavy pruning is not necessary because vegetative growth is regulated by reduced rainfall in the dry season. In addition, some of the TSH varieties have low vigour *e.g.* TSH 919. Seedling populations, as used on this farm, can result partly from selfing of self-incompatible clones through mixed pollination, which will possibly contribute to the low vigour of the trees.

Plant nutrition
The fertilising regimes followed in the early years were based on leaf analysis results. However, it is now calculated that 120 % of the minerals removed by the economic yield *i.e.* dry bean production is returned to the system. Based on a yield of 1.5 tonnes ha-^{1}, this is equivalent in kg ha-^{1} to 40 N, 8 P, 18 K, 2 Ca and 6 Mg. Nitrogen nutrition is carefully monitored to avoid excessive vegetative growth, which will increase pruning costs. Leaf litter development has been quite extensive due to the very high density planting, and consequently, nitrogen cycling from the litter will be significant. Fertilisers are applied twice per year, and in dry years additional N is applied at the start of the dry season to encourage leaf flushing

Field sanitation
The loss of pods to black pod disease is estimated at between 5 -10%. Cultural control through optimum field sanitation, adequate drainage and light penetration has kept black pod disease at low levels. Drains of the dimensions 60 x 30 x 30 cm have been established at 1.2 km ha-^{1} and are cleared annually. No fungicidal sprays are used. Weed growth is almost absent due to the deep leaf litter layer present in the fields and the interlocked canopies. When practised, weed control is done manually and through spot spraying of hardy weeds.

Pollination enhancement
The microclimate created under the interlocked canopies helps to increase midge (*Ceratopogonidae*) population sizes and activities. The discarded pods are rearranged over the entire field and act as water receptacles, which are essential for midge reproduction (Young 1986). This action replaces the role of banana pseudostems in intercropped cocoa fields. Insecticidal sprays are rarely applied; recent outbreaks of pink mealy bug (*Maconellicoccus hirsutus*) are being treated with three exotic natural enemies.

General considerations

HDP technology is an alternative approach to significantly increase yield per unit area of land and involves higher levels of crop management. Its application within the context of a cocoa orchard system was discussed by Posnette (1982) and Mooleedhar (1991). Some of the important principles noted by these authors are discussed in relation to the use of HDP in Trinidad.

Role of the variety and plant husbandry practices
Varieties used in HDP will exert a considerable influence on the productivity and profitability of these systems through their inherent yield, precocity, vigour, drought tolerance and disease resistance. The results of several trials (Atanda and Jacob 1974; Glendinning 1996) showed that the yield potential of superior hybrid varieties is close to 2000 kg ha-^{1} and can exceptionally exceed 3000 kg ha-^{1} (Lockwood and Pang 1992)

Sustaining yields of a vigorous variety beyond the first ten years of production will require manipulation of vegetative growth (pruning), adequate moisture regimes and optimum fertilising and tree health.

The performance of the TSH clones and seedlings in HDP in Trinidad has demonstrated their inherent capability for productivity and adaptability under optimum management. Their ability to yield well with the absence of shade and the application of fertilisers is now well established. The use of seedlings will promote a better rooting system than rooted cuttings (normally adopted for clones in Trinidad) for the expected high inter-tree competition of an HDP system. Additionally, seedlings will better tolerate dry season conditions than rooted cuttings, which may have decreased plant vigour. This advantage may also be obtained from grafted clones, which will also possess superior yielding ability. HDP reduces the size of the tree and seems to result in a favourable distribution of assimilates between the reproductive and vegetative parts. Hadley and Yapp (1992) noted that trunk girth (size) is not a good indicator of yield efficiency because genotypes show differences in partitioning dry matter between trunk and canopy. Studies generally link clone vigour and planting density (Lockwood and Pang 1996; Efron *et al.* 2003) and recommend low vigour clones for HDP.

TSH varieties are precocious and will start bearing in three years. Yields of 220 and 345 kg ha^{-1} respectively were obtained for TSH 1188 in the third and fourth year of a breeding trial (Shripat 2001). Achieving early cropping is a desirable feature of TSH varieties, which ensures revenue generation in the early years of establishment.

Unusually dry years can increase the death rate of trees in HDP. The farm described above has an annual tree death rate of 0.1%. However this reached 0.9% in 2003 due to an extreme dry season (124 mm of rainfall between January to May 2003). Irrigation systems will add considerably to the operating costs of HDP, therefore the drought tolerance of the variety grown becomes crucial under Trinidad conditions. Antwi (1993) demonstrated a large variation in drought response among the TSH cultivars. TSH 919, 1076 and 1220 were ranked as having desirable drought tolerance. TSH 919 demonstrated an additional adaptation of leaf rolling towards the abaxial surface. The issue of long-term yield sustainability, with no shade and unusual dry weather patterns is relevant in this context.

The TSHs have shown good levels of tolerance/resistance to black pod disease (Shripat 1993; Thevénin and Umaharan 2002). Sreenivasan (1975) reported TSH 1188 to be resistant to this disease. The higher levels of management associated with HDP have a positive effect on the TSHs in coping with black pod disease. This results from greater light penetration, and reduced field humidity due to pruning and the absence of overhead shade, which is unfavourable for survival of the pathogen. The varieties are better able to express disease resistance when inoculum pressure is low.

Economic considerations

The yields realised under the current systems of production in Trinidad have generally made cocoa farming uneconomic (Mooleedhar 1991). The cocoa industry has to rely on production systems that will significantly improve cocoa productivity and yields. Trinidad still has both competitive and comparative advantages in the production of fine or flavour cocoa. This cocoa is niche marketed and enjoys a premium price of approximately US $2800 per tonne.

Despite its demonstrated success, the adoption of HDP technology has remained at very low levels among small farmers in Trinidad.

The reasons are as follows:

(a) The average age of the small farmer population is approximately 58 years. This contributes to an unwillingness in making investments. LDP systems require lower levels of inputs than HDP as seen in Table 3.
(b) An LDP system facilitates intercropping which provides continuous sources of income for small farmers.

Table 3 shows the variable costs (VC) associated with three systems of cocoa production in Trinidad. The profits of LDP mainly represent returns to the farmers' own labour. Improved LDP uses inputs with better agronomy and management resulting in much higher yields and revenue. The highest VC of US $1699 per ha associated with HDP is indicative of the high levels of management required for its operation. In HDP, however, two areas of significant cost reduction are in weed management and black pod disease control. Table 4 illustrates the efficiency gains in moving from a low input technology to HDP. The benefit : cost ratio shows the marginal improvement in revenue and increases in cost associated with making these technology changes. This change is 39% in moving from typical low input to improved LDP and 100% from typical low input to HDP. The benefit in moving from improved LDP to HDP is 76%. These changes are substantial enough to recommend HDP both on a technical and economic basis. However, this costing also indicates that HDP requires higher levels of both inputs and management to be successful.

Conclusion

The production of cocoa in Trinidad can be successfully done using HDP. The TSHs, particularly TSH 919, are suitable for use in HDP due to their yield potential, while having fine or flavour attributes. However, the use of HDP requires a high level of management and inputs to be sustainable. It will be an important system in a modern cocoa economy involving entrepreneurial farmers. Its relevance for Trinidad is critical where agricultural lands are limited and threatened by other uses. A close study of yields and performance on the case study farm for ten or more years is needed to assess long-term sustainability.

Table 3. Variable costs of production (US $) for Low Density Planting (LDP), Improved LDP (ImpLDP) and High Density Planting (HDP) systems of cocoa production in Trinidad

		LDP	LDP (Imp)	HDP
Plant population	pl per ha	700	700	3000
Spacing	m	3.6 x 3.6	3.6 x 3.6	1.8 x1.8
Average Yield	Kg/ha	200	650	1800

	Units	Quantity LDP	LDP Cost ($)	Quantity LDP (Imp)	LDP (Imp) Cost ($)	Quantity HDP	HDP Cost ($)
VARIABLE COST							
Labour	Man day (md)						
Weed Control	md	8	76	10.5	100	3	29
Fertilising	md	Nil	-	3	29	3.75	36
Fungicide	md	Nil	-	2.4	34	Nil	Nil
Pruning	Per tree	Nil	-	.08/tree	56	.05/tree	150
Drainage	md	Nil	-	3	29	3	29
Harvesting	md	8	76	28	267	65	619
Fermentation / drying	md	2	19	6.5	62	17	162
Materials							
Fertiliser	Kg	Nil	-	360	102	1500	428
Fungicide	Kg	Nil	-	5	35	0	0
Weedicide	litre	Nil	-	2.5	10	1	10
Others							
Land tax	Ha	-	4	1	4	-	4
Maintenance (equipt)		-	-	-	24	-	90
Miscellaneous (bags etc)		-	4	-	8	-	16
Transport							
Fertiliser	Kg	-	-	-	8	-	18
Processed cocoa	Kg	0.06	12	0.06	39	0.06	108
Total Variable Cost	US$	-	191	-	807	-	1699
Revenue	Kg	1.9	380	1.9	1235	1.9	3420
Profit	US$	-	189	-	428	-	1721
Revenue Cost Ratio		-	1.98	-	1.53	-	2.0

Table 4. Effect of production systems on yields (kg ha^{-1}) and returns (US $ ha^{-1}) to cocoa cultivation in Trinidad

System of cocoa production	Cocoa yields (kg ha-1)	Gross revenue (US $/kg) over low input LDP [and improved LDP]	Variable cost (US $/ha) of improved management	Benefit /cost ratio
Typical low input farm	200	Nil	Nil	0
Improved LDP farm	650	855	616	1.39
HDP farm	1800	3040 [2185]	1508 [892]	2.01 [2.45]

Values in parenthesis [ ] represent returns of an HDP farm over improved LDP

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Farmer-Researcher Participatory On-farm Selection of Improved Cocoa Varieties: the Nigerian Experience

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Abstract

Selection of superior progenies for release and distribution to farmers is often done on-station within researcher-managed breeding trials. From an on-going study on the collection of germplasm in farmers' plots carried out in all three cocoa growing regions in Nigeria, several findings which have great implications for a successful breeding programme and ultimate adoption of research results by farmers were obtained.

From interactions with more than 120 farmers in over 200 farm units, we found that although farmers show some interest in improved materials distributed from research stations, they often select outstanding trees on their earlier established plantations as the source of planting materials for new plantings and rehabilitation of old plots. Farmers were able to identify outstanding trees on their farms, *e.g.* for yield and black pod resistance and low vigour trees amenable to high density planting, indicating their ability to monitor and select proven individual trees. Analysis of their selection criteria shows that the most important factor for the farmers is the tree yielding capacity, irrespective of its disease resistance potential. The yield capacity is defined in two terms: number of fruits produced per harvest round within a season and number of harvest rounds per fruiting year. Farmers tend to select trees that produce ripe pods throughout the year, and particularly those that produce appreciable number of pods during the off-season (dry months of January to March). The elimination of spraying costs and provision of some income at this time outweigh the reduced bean size, in the farmers' consideration.

In terms of selection for black pod resistant materials, farmers were able to identify less susceptible trees. They identified related traits, such as very thin pod husk and medium rugosity of the pod wall. In the farmers' opinion, local materials from the West African Amelonado population were more resistant to *Phytophthora* pod rot than the Amazon cocoa, though the latter was higher yielding. Farmers were able to differentiate between Amazon and Amelonado cocoa using phenotypic characteristics such as pod size and shape, pod content (pod-filling) and tree vigour.

The implication of these findings is that there is a need for a shift in breeding strategies to incorporate 'expert' farmers in a more dynamic selection process, making use of the potential of the genetic resources conserved in farmers' plots.

Introduction

The cacao tree, *Theobroma cacao* L., which has become the most important cash crop in West Africa and component of sustainable livelihood for smallholder farming families in growing areas, was first introduced into Nigeria in 1875. Initial planting materials were mainly of Amelonado, brought from Fernando Po. The Amelonado cocoa (with green pods), was introduced into the West African coastal area by the Portuguese and Spaniards in 1722 from Bahia in Brazil. This formed the base of the popular "West African Amelonado" population (in which introgression from Trinitario

is more evident), present in most parts of West Africa before the later introduction of Upper Amazon materials from Trinidad in 1944. In Nigeria, records show that trees with red pods (Trinitario from British West Indies) were also present around 1900, *e.g.* the Agege cocoa variety, originating from Lagos Botanical Garden. The Jamaican curator of the Botanic Garden had presumably brought the red-podded Trinitario type into Nigeria. Organised research began in early 1930s by Voelcker with the sole aim of development and release of improved planting materials. This effort was later taken over by an inter-territorial research thrust with the establishment of West Africa Cocoa Research Institute (WACRI) with headquarters in Tafo, Ghana and a substation in Ibadan, the present headquarters of the Cocoa Research Institute of Nigeria (CRIN).

Although several varieties have been developed in research stations, use has been restricted because farmers continue to use their own trees as source of materials for new plantings and rehabilitation (Eskes 2000). The type and source of planting materials used by farmers are very important factors in achieving a sustainable cocoa production system. At present, research activities for the selection of superior progenies to be released and distributed to farmers is often done on-station within researcher-managed breeding trials (progeny trials, hybrid selection, clonal trials, early screening *etc.*). This is often based on the researcher's perception of farmers' needs.

The objective of the work reported here was to document farmers' selection criteria and practices in a farmer-researcher participatory selection activity carried out on farmers' fields. This study was carried out within an ongoing programme on the assessment of genetic diversity in cacao populations in Nigeria. Two major ideas behind this study were: (a) genotypes with improved traits are available in farmers' fields, and (b) farmers' genetic resources are more dynamic than that in on-station field genebanks in terms of evolution of genetic diversity and could provide an alternative, and possibly 'better', source to select new cultivars than the genebanks available on-station.

Materials and Methods

Study area

Germplasm materials were collected from farmers' plots across all three cocoa growing ecologies in Nigeria (Figure 1). Having gained access to farms following various customs within the cultural setting of each location, essential information on farmers' selection criteria and practices were obtained through a rather more informal than formal interaction with farmers. The information collected included husbandry practices (spacing, pruning *etc.*), source of planting materials, disease and pest problems and management, yield, changing climate, local practices, traits of interest, criteria for selecting outstanding trees, relevance of research *etc.*

Germplasm collection was done based on:

a. Farmer-led selection of:

- Interesting and supposedly outstanding materials ("farmers' best trees"). These were trees identified as interesting by the farmers (generally for high yield or low pod rot incidence), as well as trees that the farmers use as "mother trees", that is, trees from which pods are collected to raise seedlings for new plantings.
- Undesirable trees ("farmers' worst trees"). These are trees he has observed to be very susceptible to pod rot and low yielding.

b. Researcher-influenced joint selection of trees:
This was based on researchers' perception of apparent relative yielding capacity and medium vigour in relation to neighbouring trees, number of beans per pod, pod shape and pod colour.

Results and discussion

Several findings having implications for successful breeding programmes and ultimate adoption of research results by farmers were obtained.

Farmers' Practices
Although farmers show some interest in improved materials distributed from research stations, they often consciously select outstanding trees on their earlier established plantations as the source of planting materials for new plantings and rehabilitation of old plots. Most farmers show preference for such selected trees, on their own or neighbour's farm, as the source of planting materials, rather than the young seedlings distributed by government agencies. They claimed that such selected trees were often found to replicate the interesting traits for which they were selected in derived progenies and preferred to use this material rather than be in a less predictable situation with plantations established with materials from "untested and unproven" government source. This was a sort of risk aversion strategy, particularly for such a long gestation crop as cacao. This is based on their consistent observations and evaluation of such trees' performance relative to others on their farms. This attitude holds great promise for variety selection in an on-farm trial.

We found that farmers continue to adapt recommended husbandry practices, such as tree spacing, pruning, fungicide application time and frequencies to their situation. They claimed that their methods were adopted after several 'trials and errors'. Thurston (1992) had observed this as the basis of several local practices in disease management.

Farmers' knowledge and selection criteria
Farmers were able to identify some very outstanding trees on their farms, indicating their ability to monitor and select proven individual trees. In the farmer's selection criteria, yield capacity of the tree is the most important factor, irrespective of its disease resistance potential. The yield capacity was defined in two terms: number of fruits produced per harvest round within a season and number of harvest rounds per fruiting year. Farmers tend to select trees that produce in all harvesting rounds of the year, and particularly those that produce appreciable number of pods during the off-season (dry months of January to March). Elimination of spraying costs and provision of some income at this time outweigh the reduced bean size, in the farmers' consideration.

Of the cocoa types available in the field, all the farmers interviewed across the country pointed to the red-podded trees (probably Trinitario) as the highest yielding type, rather than Amazon or local Amelonado. In Ondo State, in the Southwest of the country, this phenomenon has however assumed a negative impact on the further propagation of this cocoa type. In the local "parlance", it is exaggerated that "the red-podded tree bears, even on its roots" and that "the tree's productivity is such that it takes the owner's life". As a result, in this part of the country where farm sizes are relatively large (average: > 1.5 ha per farmer), most farmers allow only a few stands of red-podded (Trinitario materials) on their farm and minimise their use as mother trees for new plantings as compared to high yielding green-podded trees. The huge amount of labour required to maintain the tree's high yield in terms of spraying and harvesting, which is often more than what a farmer's family can afford,

was the major reason given for this assertion. On the other hand, in marginal cocoa growing areas, where farm sizes are usually small (average: < 1.0 ha per farmer), most farmers select such red-podded trees as mother plants for further plantings. This is because of the expected higher yields obtainable with such trees on the small land area cultivated. However, Trinitario materials are not planted exclusively on these farms because the farmers also appreciate the need to maintain genetic diversity on their farms.

Resistance to black pod was the next important factor. They were able to identify less infected trees, although there was always very few of these (*i.e.* 2 to 6 per farmer plot with an average size of 1.2 ha). Farmers identified the following traits to be associated with less infected trees (pods): very thin pod husk (easily breakable with a slight pressure), medium rugosity and medium size. Some farmers mentioned that most trees carrying pods with red pigmentation were not easily infected. However, when such pods are infected, the rate of infection spread on pods was faster than in any other variety. In the farmers' opinion, local materials of the West African Amelonado population were less infected by *Phytophthora* pod rot than the Amazon cocoa types. However, they recognised the latter as higher yielding.

Farmers were able to differentiate between Amazon and Amelonado cocoa using phenotypic characteristics such as pod size and shape, pod content and tree vigour. Farmers prefer productive trees with medium sized pods with a large number of beans per pod (good pod-filling) rather than vigorous trees with large size pods that may have less beans per pod.

We found that although Amazon cocoa is the predominant type now grown, farmers still identify the following favourable traits of Amelonado:

- 'easy response' to black pod control (appreciable yield recorded with 0 – 2 spray applications unlike Amazon cocoa which requires at least six applications during the main production season);
- low vigour (less space and amenability to higher plant population per hectare);
- higher bean/pod volume ratio (less mucilage and placenta content);
- Longer productive years, that is, more productive in old age than earlier years unlike Amazon, which is more productive in earlier years than old age;

Disadvantages of Amelonado identified by the farmers include:

- long juvenile period (late bearing);
- lower pod yield;
- susceptibility to mirid attack during dry season;
- single harvest period.

Farmers' "Ideal Cocoa Variety"

With a very good knowledge of the advantages and disadvantages of the cocoa populations present, the farmers have come to a definition of their ideal cocoa variety: an "Amelonado type of Amazon cocoa" variety combining the following traits: high pod yield, high bean yield per pod (not less than 45), year-round production (in main plus minor season), resistance to *Phytophthora* pod rot, and medium vigour (for higher plant population/hectare). This ideotype preferred by the farmer can be termed "Amelzon" or "Amazonado" cocoa, a hybrid combining the excellent qualities of both Upper Amazon and Amelonado populations.

Conclusions

Findings from this study showed that farmers' selection criteria are not altogether different from those of the researchers. A shift to a new paradigm in cocoa breeding and selection, where both could work together in a 'farmer-led but researcher-driven' selection and breeding activities, is hereby suggested. The farmers attitude of risk

aversion, in using only trees that have met their own criteria, rather than from the formal seedlings distribution system, of which they are unsure, is a good impetus for on-farm variety testing, as envisaged in the new CFC/ICCO/IPGRI project on "Cocoa Productivity and Quality Improvement: a Participatory Approach". In this case, farmers' involvement in selection from an array of variability which has been created by breeders from several crosses made on-station would help to feed several interesting cultivars into the informal planting material distribution system. Clonal propagation of materials selected by farmers and by researchers in farmers' fields should be encouraged. These could be compared by the farmers with existing hybrid varieties. Such selected trees should also be incorporated in further breeding and selection activities. This participatory approach has been used in crops such as finger-millet (Gowda *et al.* 2000) and barley (Ceccarelli *et al.* 1997) to facilitate a high rate of variety adoption and significantly reduce the number of years required in variety identification and adoption.

It should be noted also that disease resistance, *per se*, was not a major selection criterion to the farmers, but only secondary to the yielding ability of the cacao tree. The farmers' main concern is yield and avoidance of crop failure. The farmers have some understanding of the concept of resistance, and its heritability. They know quite well that some trees are less infected than others and expect such traits in the offspring. This is the basis of selecting such trees as "mother trees" for new plantings. Understanding of this concept of heritability is particularly demonstrated in their choice of heavy pod yielding trees as "mother trees" for new plantings. For more precise selection however, the researchers have the exclusive role of evaluating further such less infected trees by using available inoculation techniques. A consequence of this for breeding could be that emphasis should not be put on disease resistance at the expense of yield. Yield, though a complex trait, should remain the chief goal of a cocoa-breeding programme, while selection for a high level of resistance should be a very important complementary objective, as this has implications for reducing crop failure due to disease attack, a major production constraint and cost item in the farm economics.

From this study, it has been shown that adopting this complementary initiative, entailing farmer-researcher interaction, would ensure sustainable cocoa production adapted to farmer-managed situations. The farmers should be seen as true partners and involved in all stages of the selection process, which should be carried out under a low external input agriculture, as prevalent here. They should also be exposed to scientific information that could help them make better decisions adapted to environmental and husbandry situations.

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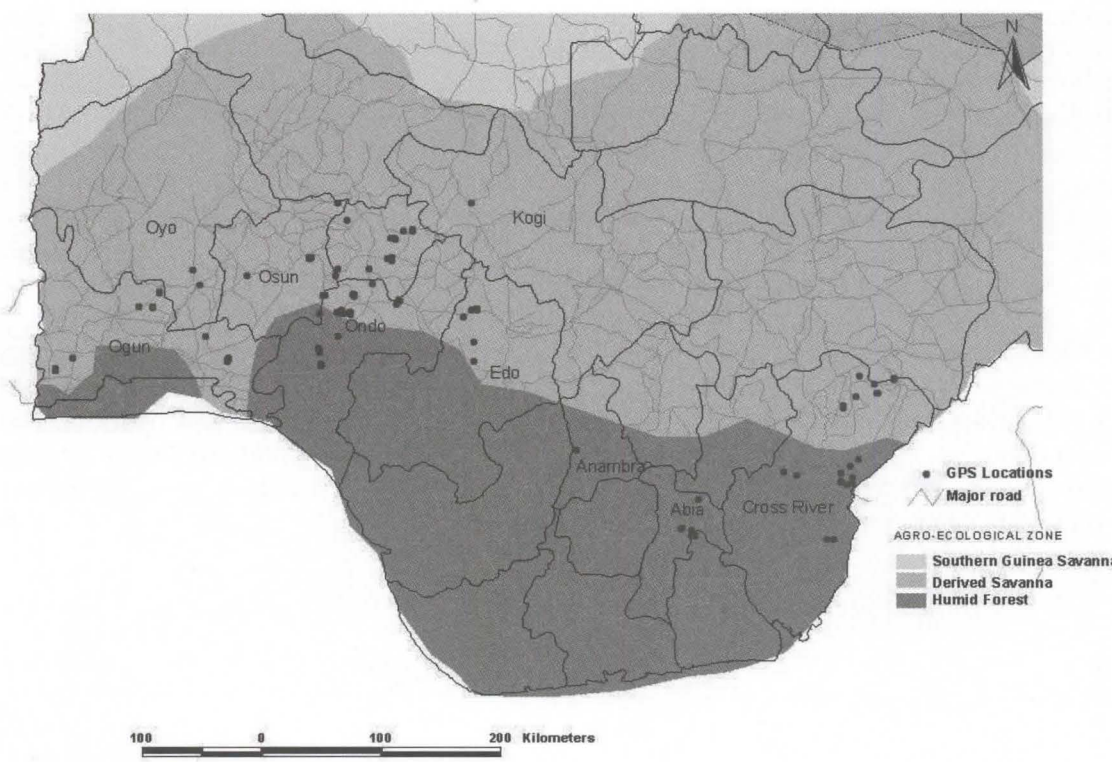
Figure 1. Map showing cacao germplasm collection sites in cacao growing areas of Nigeria.



Perspectives on Rapid Vegetative Multiplication for Orthotropic Scion and Rootstock Varieties of Cocoa

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Abstract

The vast majority of both available information and cultural practices related to cocoa cloning focuses on plagiotropic vegetative material (mature fan branches) and ignores orthotropic material (juvenile chupons). Improved knowledge sharing related to the potentials of orthotropic scions and rootstocks is needed to help not just growers but also researchers and extensionists. Orthotropic scion and rootstock cloning offers potential advantages for growers such as seedling-like architecture, reduced shape pruning, improvement in tree anchorage and nutrient uptake and aspects of drought avoidance. Orthotropic rootstock clones may also be the preferred material used for development of superior rootstock clones in cocoa, such as those having "dwarfing" characteristics. Breeding, extension, and nursery programmes can take advantage of the juvenile material to speed up evaluation, selection, distribution and multiplication. Here we demonstrate, from a review of the widely dispersed past and current research, some of these and other possibilities and realities for clonal orthotropic scions and rootstocks in cocoa.

Historic perspective on orthotropic and plagiotropic advantages and disadvantages

> *'The problem is to ensure a constant supply of "hard" chupons of small diameter, which are uncommon under field conditions. This can almost certainly be solved in a budwood nursery by keeping chupon stools of the required scion types hard pruned…' -F.J. Pound (1935)*
>
> *'I think that it is now accepted that the future of cocoa- perhaps a very distant future yet- is the high yielding clone, the job of the present is to produce it.'-F.J. Pound (1945)*

The dimorphic growth of *Theobroma cacao* influences the architecture of the plant derived from seedlings that comprise approximately 95% of the trees in cultivation, as well as the remaining clonal plantings. Cocoa's characteristic "Nozerman" model of tree architecture arises from alternating juvenile, orthotropic (vertical) growth that is interrupted abruptly at the jorquette. From this jorquette (fan) arises the source of the mature, plagiotropic (lateral) growth of the canopy. A secondary orthotropic meristem, subtending a jorquette, emerges to repeat, in a step-wise manner, the vertical growth that ends, more or less, within a metre at the new topmost jorquette. In this manner, the canopy is raised at controlled increments, and the mature seminal tree architecture may consist of several levels of these whorls of fan branches (Cuatrecasas 1964; Greathouse *et al.* 1969).

The first research efforts into the vegetative propagation of cocoa noted these stark differences in potential source material from ortets (Harland and Parga 1924; Pyke 1933; Cheesman 1935). Plagiotropic scion material from the mature canopy, when propagated, results in a sprawling, bush-like architecture arising from bud

break of plagiotropic axillary meristems. In contrast, orthotropic material from chupons produce scions with initial upright orthotropic growth, jorquette formation and architecture similar to that of the seminal tree, be it of more variable height.

Root systems of rooted plagiotropic and orthotropic material have also been a topic of note since early research into vegetative propagation (Cheesman 1933; Pyke 1933). Superficial fibrous roots explore nutrient-rich upper soil horizons where many nutrients such as phosphorus are most available in organic rich material. Superficial root systems support rapid growth during establishment in optimal environments. Taproot systems, whether seminal or adventitious, also consist of superficial roots and are considered to be important for 1) strong tree anchorage, and 2) moisture scavenging at soil depth during periods of superficial soil horizon moisture stress. While less of a concern on well-maintained research plots, many growers prefer trees with taproot systems. This is of particular importance for hillside plantings.

Initial observations suggested plagiotropic adventitious root systems were superficial and fibrous, and that orthotropic material formed adventitious taproot systems (Cheesman 1935). Later, investigations of established plants reported that plagiotropic rooted material may later on form taproots or vertical brace roots (Cheesman 1936; Bowman 1948). Fordham (1973) displays a diagram in which a plagiotropic rooted cutting of the clone ICS 1 is shown to definitely lack a taproot system. Following these informative but rather incomplete studies, two exhaustive works thoroughly established the inconsistency of plagiotropic material in forming taproots while orthotropic material consistently did so (Van Himme 1959; Charrier 1969). Our investigations with orthotropic, single-leaf (node), softwood rooted cuttings have demonstrated that highly gravitropic taproots form during rooting, and between one to four, or occasionally more, taproots firmly establish during one year when grown in the greenhouse. We feel these orthotropic rooted cuttings will be shown to provide better anchorage and to allow improved escape from shallow soil horizon moisture stress than plagiotropic root systems. Orthotropic stem material is likely to become the choice material for mass multiplication of "dwarfing rootstock" clones for cocoa, once these have been developed.

Attempts to overcome plagiotropic shortcomings

Following the general consensus as to the selection of source material used for vegetative propagation, emphasis was then placed on cultural methods to overcome the 'shortcomings' of plagiotropic clones. Numerous techniques have been evaluated in efforts to determine the most productive shape pruning management for the troublesome bush-like growth habit of plagiotropic clones (Murray, 1961; Ramadasan *et al.* 1978). While shape pruning is a straightforward technique for overcoming poor plagiotropic habit, pruning of establishing trees is wasteful of carbon resources and labour. Furthermore, effective formation pruning and tree management of canopies with wide branching angles that grow low to the ground (such as many of SCA 6 progenies) is difficult. Therefore, plagiotropic cocoa scions should be developed that have more acute branching angles and an "erect" growth habit. These will be more acceptable to growers. In contrast, orthotropic scions, displaying either horizontal or erect canopy growth, would require less pruning and consequently less labour during establishment.

Current extension advice given to growers in Trinidad suggests that plagiotropic clones should be established and tended in the field for a number of years, and adventitious basal chupons should then be nursed to establish an orthotropic tree, that is expected to subsequently develop a taproot system (Mooleedhar 2000). This sidesteps the issue of developing productive orthotropic clonal gardens while recognising the inherent advantages of orthotropic clonal cocoa off of research station experimental plots and in growers' fields. However, this wastes

carbon resources and is therefore not an efficient technique to establish orthotropic clones. The authors have observed that even after six years in the field, many plagiotropic clones had still not developed chupons, which leaves the planting in an unsatisfactory plagiotropic/orthotropic mosaic. Simply stated, it is recommendable to begin with orthotropic scions if that is the desired end result.

Efforts to promote orthotropic scion material in stock plants

Relatively few efforts have been made to establish orthotropic clonal gardens for use as scion and/or rooting material. Historically, most established clones have been propagated exclusively by means of plagiotropic fan material. Expeditions made for collecting wild accessions returned with plagiotropic green wood for grafting (Pound 1938; Pound 1943). Researchers have taken turns discrediting orthotropic vegetative propagation in cocoa over the century following the first account of grafting by approach (inarched) on seedling stocks by Hart in 1898, patch budding in cocoa by Harris and Heyl in the early 1900's, and the seventy-plus year history of rooted cuttings initiated with the work of E.E. Pyke. Many reports mention orthotropic material, principally collected as basal chupons (water sprouts), as limited in availability, poor in rooting and/or establishment and discounted it as inappropriate for vegetative propagation. (Archibald 1955; Urquhart 1961; Cuatrecasas 1964). This has led to the current situation where plagiotropic material has become exclusively regarded as that used when vegetatively propagating cocoa.

Cultural strategies have been tested to promote the growth of orthotropic material in clonal gardens with mixed success. In East Java, orthotropic budwood gardens in the form of adult trees that are trimmed regularly to produce chupons, have been used to multiply commercial DR clones ("Edelcacao") by budding in the field on seedling rootstock (Eskes, personal communication). Promotion of chupons from mature trees produced variable amounts of orthotropic material for rooting depending on the mother trees and on the method used (Bertrand and Agbodjan 1989). Bertrand and Dupois (1992) experimented with orthotropic budwood gardens and used arching of orthotropic rooted cuttings to stimulate chupon growth.

Bending of plagiotropic cuttings and of seedlings has also been demonstrated to produce chupons (Amefia *et al.* 1985; Cilas *et al.* 1985). Seedlings with up to five years in the greenhouse have been demonstrated to produce repeated harvests of chupons when arched to horizontal (Glicenstein *et al.* 1990).

In selection and breeding programmes, where segregating populations are cloned as early as possible, particularly those foregoing the seedling evaluation stage, orthotropic material is more readily available and may then be more easily introduced into horticultural nursery multiplication practices (Efron 1999; Efron *et al.* 2000).

Performance of orthotropic rooted cuttings and grafted scions

Results of a series of field experiments planted at River Estate, Trinidad in 1937/38 through 1949, known as CRB 1 to 16, were among the first to indicate performance of cloned material in cocoa (Cope 1949). In several experiments, rooted cuttings were deemed to perform better than budded seedlings. While limited by single degree of freedom comparisons, orthotropic and plagiotropic treatments were included in the design of many of the experiments (Cheesman 1941). Dodds and Cope (1951) wrote, after several years of preliminary evaluation, that generally 'there are no intrinsic differences in the yield potentialities of fan and chupon material'. In Malaysia, orthotropic budded clones appeared to be slightly inferior to plagiotropic

buddings, but this difference was barely significant and obscured by significant interactions between clones and propagation methods (Pang *et al.* 1994).

The perceived difficulty in obtaining orthotropic material from a conventional clonal garden appears often to be the main reason for concentrating on the use of plagiotropic material in all clonal evaluations. However, it is interesting to note that current thinking in Trinidad emphasises the local preference for orthotropic material due to practical plantation management concerns (see also above).

Efron (1998) and Efron (1999) have demonstrated that cultural practices, environment and genetic components control height of jorquette in orthotropic budded seedlings. Our research with orthotropic rooted cuttings also establishes a cultural aspect for the control of jorquette height. Rooted cuttings taken from the base of a chupon yield low-jorquetting trees, while trees arising from cuttings proximate to the chupon apex demonstrate more variable but significantly greater mean first jorquette height (unpublished results). If higher jorquetting architectures are desired, the first fan bud may easily be pruned early on to release subtending axillary meristems for continued orthotropic growth.

Outlook for development of intensive, high density orthotropic clonal gardens

Experience with somatic embryo derived cocoa clones has offered us the opportunity to investigate the development of an integrated orthotropic clonal garden and rooted cutting system. Somatic embryos lend themselves well to this endeavour due to their seedling-like growth when acclimatised in the greenhouse (Li *et al.* 1998; Guiltinan *et al.* 2000; Maximova *et al.* 2002; Traore 2000; Traore *et al.* 2003; Lambert *et al.* 2000). They may be repeatedly harvested at the softwood stage by strong heading cuts as little as three months after acclimatisation. Alternatively they may be left until phase change at the semi-hardwood stage (nine to twelve months in greenhouse), the fan branches pruned hard, and the trunk arched to horizontal. These procedures release first axillary chupons, and later adventitiously derived chupons from the cork cambium. Exclusively orthotropic stock plants may be readily developed and repeatedly harvested if well managed in the nursery. Both techniques make productive stock plants in addition to making efficient use of nursery space. Either technique may be appropriate for establishing orthotropic clonal gardens of exclusively orthotropic-producing ortets, and tissue culture need not be a necessary step in the process. Detailed protocols for these methods can be found on the following website or by contacting the authors:
(*http://guiltinanlab.cas.psu.edu/Research/Cocoa/tissue_culture.htm*).

We also suggest the establishment of orthotropic clonal gardens from standard horticultural practices. The limited numbers of chupons from desired mature clones would first be harvested. This material should serve not as plants for field plantings but rather as nursery stock for establishing exclusively orthotropic stock plants and later orthotropic clonal gardens. These stock plants can be easily multiplied in the nursery by rooting of single leaf softwood stem cuttings and/or by budding/grafting. A grafting strategy may increase the rate of clonal garden establishment in comparison to the use of rooted cuttings, though if nurseries were to use grafted seedlings as ortets care should be taken to ensure that rootstocks are not mistakenly propagated in place of scions,

Conclusion

What is currently held as common knowledge regarding the vegetative propagation in cocoa has resulted from many historically significant factors. This brief work attempts

to join together, in a cohesive manner, results from over a century's worth of studies and observations on the topic from diverse geographical areas to forward the newly re-emerging concept of orthotropic vegetative propagation in cocoa. Productive, quality trees are the objective of researchers and extensionists and the desire of growers. Access to alternatives when planting, renovating or replanting are essential to ensure that the grower may have a choice that best fits his or her situation.

Alternatives for orthotropic scion and/or rootstock development (and potential drawbacks) should be better communicated to parties conducting this avenue of research. In addition, the basic horticultural methods used for producing large, sustained quantities of orthotropic materials should be introduced into participatory extension programmes as potential options for clonal garden establishment and nursery management. Also, rigorous replicated experiments should be established that include orthotropic versus plagiotropic treatment effects on scion and/or rootstock establishment, management and performance to shed further light on this old and important but none-the-less inadequately studied topic.

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WORKSHOP PROGRAMME

Cocoa Breeding for Improved Production Systems

19-21 October 2003, Hotel Miklin, Accra, Ghana

Sunday 19 0ctober

10.00-17.00 Meeting of the "Cocoa Genomics Study Group" (Hotel Miklin)
16.00-18.00 Workshop registration

Opening Session (Together with INCOPED)
Convener: Dr. Laud Ollennu (CRIG) *Chairman: Dr. Roy Appiah (CRIG)*

18.00 Introduction by the Convener
18.05 Response of the Chairman
18.15 Welcome address and official opening of the workshop (Chief Executive of the Ghana Cocobod)
18.30 Welcome addresses by INCOPED, INGENIC and COPAL
19.00 *Invited speaker*: Uilson Lopes (CEPEC/CEPLAC) Participatory Breeding for Witches' Broom Resistance in Bahia, Brazil – a Historic Retrospective

19.30 *Joint INCOPED/INGENIC welcome cocktail (offered by the Cocobod)*

Monday 20 0ctober

Session 1. Introduction and invited speaker
Chairperson: Yaw Adu-Ampomah, Secretary: Kolawole Badaru

8.30 Introductory notes and workshop objectives (Bertus Eskes, INGENIC)
8.45 *Invited speaker*: Yoel Efron *et al.* (CCRI): Cocoa breeding for improved production – the PNG experience

Session 2. How to select superior mother trees in heterogeneous progenies for more productive clone or hybrid varieties?
Chairperson: Rob Lockwood, Secretary: Peter Aikpokpodion

10.00 How to improve the efficiency of individual cocoa tree selection in progeny trials? (Christian Cilas, CIRAD)
10.15 Assessment of the yield of individual cacao trees in four field trials (Boamah Adomako and Yaw Adu-Ampomah, CRIG)
10.30 Analysis of factors affecting yield and yield variability in the SG2 cocoa hybrid in Papua New Guinea (Yoel Efron *et al.,* CCRI)
10.45 Individual tree variation and selection: experience from Côte d'Ivoire (Olivier Sounigo *et al.,* CIRAD/CNRA)
11.00 Comparison of values as clones and values as progenitor for yield, vigour and yield efficiency: experience from Côte d'Ivoire (Olivier Sounigo *et al.*, CIRAD/CNRA)
11.15 Correlation between clone values observed in collection and hybrids produced by these clones, with special emphasis on yield x vigour relationship (José Luis Pires *et al.*, CEPEC/CEPLAC)
11.30 Combining ability of parental clones for high productivity of derived hybrid clones (Yoel Efron *et al.,* CCRI)

11.45 General discussion and conclusions

Session 3. Factors affecting yield x vigour relationships in cocoa
Chairperson: Uilson Lopes, Secretary: Olivier Sounigo

13.30 *Invited speaker* (Rob Lockwood, oral presentation only)
Harvest efficiency
14.15 The relationship between vigour, yield and yield efficiency in cocoa clones (Yoel Efron *et al.*, CCRI)
14.30 Physiological Characterisation of Cocoa Germplasm (Andrew Daymond and Paul Hadley, University of Reading)
14.45 Genetic effects of inter-tree competition in mixed cocoa stands on yield, vigour and cropping efficiency (Philippe Lachenaud, CIRAD)
15.00 General discussion and conclusions

16.00 *INGENIC General Assembly*

20.00 *Joint INCOPED/INGENIC dinner (Supported by COCOBOD and Masterfoods)*

Tuesday 21 0ctober

08.30 *Invited speaker* (Chris Atkinson, HRI):
Enhancing harvest index in temperate fruit tree crops through the use of dwarfing rootstocks
09.15 Review on rootstock experiences in cocoa (Rob Lockwood, oral presentation only)
09.45 A cocoa mutant with a dwarfing effect as rootstock (Yoel Efron *et al.,* CCRI)
10.00 Investigation into the use of crinkle leaf mutant of cocoa as low vigour rootstock for high density planting (Yaw Adu-Ampomah, CRIG)
10.15 General discussion and conclusions
11.00 Impacts of the micro and macro environments on the genetic gain by clonal selection in cacao (Uilson Lopes *et al.,* CEPLAC)
11.15 An evaluation of 22 clones at two locations each with three planting densities in Indonesia (Baihaqi Sitepu *et al.,* Bah Lias)
11.30 High density planting of cacao: the Trinidad and Tobago experience (Kamaldeo Maharaj, MALMR)
11.45 General discussions and conclusions

Session 6. Other perspectives related to development and multiplication of new planting materials for improved production systems
Chairperson: Jeanne N'Goran, Secretary: Kamaldeo Maharaj

14.00 Farmer-researcher participatory on-farm selection of improved cocoa varieties: the Nigerian experience (Peter Aikpokpodion *et al.,* CRIN/IITA)
14.30 Perspectives on rapid vegetative multiplication for orthotropic scion and rootstock varieties of cacao (Carter Miller and Mark Guiltinan)

Session 7. Presentation and agreement on conclusions
Chairperson: Yoel Efron, Secretary: Bertus Eskes

17.00 **Closing Session** (INGENIC, support institutions, CRIG)

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